THE UNIVERSITY OF ALBERTA

THEORETICAL AND EXPERIMENTAL STUDIES ON FROG SKELETAL MUSCLE

by

EDDY Y.-M. WONG

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF PHYSIOLOGY

EDMONTON, ALBERTA

FALL, 1972

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate of Studies and Research, for acceptance, a thesis entitled *Theoretical and Experimental Studies on Frog Skeletal Muscle* submitted by Eddy Y.-M. Wong in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

An activation-contraction model for skeletal muscle is developed based on the sliding filament theory and calcium binding. The effects on the isometric twitch and tetanus are studied by changing the model parameters which include rates of making (f) and breaking (g) of cross-bridges, amount of calcium released by a stimulus (C_0), rates of calcium binding (α) and re-uptake (β), and series stiffness (k). The characteristics of the isometric twitch are most sensitive to changes in f or β.

In a muscle with a poorly-developed sarcotubular system, both C_0 and β may be reduced. This gives a twitch which has a smaller peak tension (P_t), a lower maximum rate of tension development (dP/dt), a longer contraction time (T_c) and half-relaxation time ($T_{\frac{1}{2}r}$). A tetanus which is produced by reducing C_0 and β has a smaller maximum tension which is better maintained, a longer time to peak and a lower fusion frequency. Including a threshold in the activation mechanism intensifies the above effects and gives a sigmoid frequency-tension curve. These effects are observed experimentally. Reducing k gives a twitch with a smaller P_t and dP/dt, and a longer T_c.

The magnitudes of these effects are compared with experimental data. The effects of temperature and of a tetanus on the mechanical properties of muscle are not well enough known to have been included in the model. Experiments have been carried out to study these effects more quantitatively using the frog sartorius muscle.

P_t in the pretetanic and the maximally potentiated twitch

decreases by 49% and 22% respectively with an increase in temperature from $3\frac{1}{2}°$ to 30°C. A tetanus of a few seconds duration produces a delayed increase in P_t when temperature is below 25°C.

Following a tetanus, P_t rises nearly linearly to a maximum and decays with an approximately exponential time course. This is in agreement with the hypothesis that this phenomenon is the result of a fast-decaying inhibition and a slow-decaying potentiation. These processes have Q_{10}'s of 2.7 and 2.9. The slow process also has a half-time of about $1\frac{1}{2}$ minutes at 20°C. It is suggested that inhibition is related to Ca^{++} depletion from the release site while potentiation is associated with a decreased β.

The frequency response of the isometric sartorius muscle was determined by analysis of the tension generated during short periods of random stimulation of the muscle nerve. It resembles closely the Fourier transform of the isometric twitch, and both are well represented by the frequency response of a linear second-order system. The second-order parameters: zero-frequency gain (G_0), natural frequency (f_n) and damping ratio (ζ) provide useful descriptions of the changes in the muscle response caused by temperature changes or tetanus. For example, f_n is found to vary linearly with temperature in the range 3 to 31°C. ζ is increased following a tetanus.

At all temperatures, T_c, $T_{\frac{1}{2}r}$ and the ratio $T_{\frac{1}{2}r}/T_c$ are increased by a tetanus; but the increase in the ratio becomes marked at higher temperatures. Analysis using a linear second-order system shows that ζ depends on the ratio $T_{\frac{1}{2}r}/T_c$ rather than the absolute value of $T_{\frac{1}{2}r}$ or T_c; f_n changes in proportion to both $T_{\frac{1}{2}r}$ and T_c and is a good indicator for the 'sluggishness' of the muscle.

ACKNOWLEDGEMENTS

I am grateful to Doctor Richard B. Stein, my supervisor, for advice and encouragement throughout this work.

I would like to thank Doctor Alian C. Mannard and Mr. Sandy Brown for comments and helpful discussions.

I would also like to thank Mrs. Ortella White for typing the manuscript, and Mr. Kenneth Burt for drafting and photographic work.

This work was supported by a fellowship from the Muscular Dystrophy Association of Canada (MDA) to the author and by grants from the Medical Research Council of Canada and MDA to Doctor Stein.

To my wife, JENNY.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION.

Following the introduction of the concepts of active state and degree of activation by A.V. Hill (1949), it has been widely accepted that a muscle must be activated before it is capable of shortening or developing tension. The capacities to shorten and to develop tension in turn define the active state of the contractile component (Hill, 1951b). It is also believed that force generation in a living muscle is due to the continual making and breaking of cross-bridges between the overlapping thick and thin filaments (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954). A.F. Huxley (1957) has worked out a detailed mathematical formulation of this general nature, and has shown that it can account for many features of contraction. In his scheme, the making of cross-bridges was assumed to be the main rate-limiting factor in steady shortening, while breaking after the performance of work by a cross-bridge was relatively rapid. The results of this mathematical analysis agree well with the 'characteristic equation' formulated by A.V. Hill (1938); the latter equation relates speed and load in isotonic shortening, and was derived on the basis of muscle energetics. However, the results did not fit the transient responses, such as the change in tension following a sudden alteration of length or *vice versa* (Podolsky, Nolan and Zaveler, 1969; Huxley and Simmons, 1971). Podolsky, Nolan and Zaveler (1969) reversed the assumptions about the rate constants, attachment of cross-bridges rapid and

breakage rate limiting. In this way, they were able to fit many aspects of transient responses but not the thermal data. Recently, Huxley and Simmons (1971) applied the theories of visco-elastic behavior of high molecular weight polymers to the attachment and detachment of cross-bridges and showed that their proposal could account for the successful aspects of both of these theories.

The basis of the sliding filament model can be used to explain many phenomena in muscle and its properties. For example, the observation of Gordon, Huxley and Julian (1966) that the maximum isometric tension was related to the sarcomere length was explained if the number of interacting cross-bridges was proportional to the amount of overlapping of the filaments. The elasticity which accompanied a very small stretch in length in a passive muscle was attributed to some 'active' interaction between the filaments (Hill, 1968).

A number of hypotheses were proposed to explain the mechanism of force generation at the molecular level. Features such as ATP-splitting, calcium ions, electrostatic phenomena, myosin and actin molecule structures were usually incorporated into the hypotheses. Davies (1968) suggested that each cross-bridge shortened by folding at a number of points. Huxley (1969) proposed that the head of the myosin molecule rotated relative to the thin filament, acting as a lever which pulled the thick filament along by a link which was also part of the myosin molecule. The flexible linkage in the latter scheme enables equal force production over a wide range of interfilament separations. Thorson and White (1969) suggested a

hypothesis which related the attachment-detachment cycle of cross-bridges to the measured length-tension dynamics of the active insect fibrillar flight muscle, in which length change was small.

The sliding filament model and Hill's 'characteristic equation' are adequate if one requires only the steady-state relationship, for example, when a muscle is maximally activated. To account for the time course of a twitch or tetanus, an activation mechanism must be included. Considerable evidence indicates that the binding of calcium (Ca^{++}) by the myofilaments regulates the process of contraction and relaxation (Sandow, 1965; Weber, 1966; Ebashi, Ebashi and Kodama, 1967). Based on this concept, Julian (1969) extended the sliding filament model by including an activation mechanism which depended on a number of factors. The latter includes the rates at which calcium is bound and released, and the amount of calcium available. The time course of an isometric twitch can then be computed. However, to compute a tetanus or partially-fused twitches, summation of effects must be described. Together with the parameters in the original sliding filament model, there are no less than eight parameters, each of which can be changed independently of the others. The existence of this large number of parameters poses some interesting problems:

(1) What are the effects of changes in these parameters on the characteristics of an isometric twitch or tetanus?

(2) How closely can these changes be related to the structural differences observed in skeletal muscles? and finally,

(3) Can these changes be used to explain changes in the char-

acteristics of an isometric twitch under different physiological conditions, for example, after a tetanus?

In the event of excitation-contraction coupling in skeletal muscle, it is well known that potential change in the plasma membrane eventually leads to the release of Ca^{++}. Extracellular Ca^{++} is also required for generation of the muscle action potential (Ishiko and Sato, 1957; Edman and Grieve, 1964). Frank (1958, 1960, 1962) found that by soaking the toe muscle of the frog in a Ca^{++}-free solution for a few minutes, he could completely eliminate the potassium-induced contracture without reducing the potassium depolarization. The rate at which this inhibition developed was determined by the rate at which Ca^{++} left the extracellular space of the muscle. The contractile mechanism of the cells was, however, unaffected by this treatment because their response to an appropriate stimulus was not changed by the removal of extracellular Ca^{++}. This led him to believe that a 'loosely bound fraction' of Ca^{++} was necessary to elicit a contracture in response to depolarization of the membrane, but another source of Ca^{++}, the 'firmly bound fraction', appeared to be needed for contraction itself. The exact relationship between these two Ca^{++} fractions remains unknown. Discovery of the transverse tubules (T-tubules) with their close apposition to the sarcoplasmic reticulum (Porter, 1956, 1961; Porter and Palade, 1957; Bennett and Porter, 1953; Bennett, 1955; Anderson-Cedergren, 1959), the calcium accumulation property of the latter structure (Marsh, 1952; Ebashi, 1961a; Hasselbach, 1964), the continuity of the T-tubules with the plasma membrane (Franzini-Armstrong and Porter,

1964; Huxley, 1964; Page, 1964; Birks and Davey, 1969) and their electrical coupling (Falk and Fatt, 1964; Peachey, 1965), the local stimulation experiments of Huxley and Taylor (1958) and Podolsky and Costantin (1964), suggest that in the excitation-contraction process, potential change of the plasma membrane produces similar change in the T-tubules, and probably also in the sarcoplasmic reticulum from which Ca^{++} is then released. This hypothesis is also supported by the observation of Costantin and Podolsky (1966) and Lee, Ladinsky, Choi and Kasuya (1966) that local depolarization of the triad sacs appears to activate contraction. Activation via the T-tubules is probably more important in the fast twitch muscle, which has a well-developed internal membrane system compared to the slow muscle (Page, 1968; Flitney, 1971). This is also the 'fast' process suggested by Hill (1949) as opposed to the 'slow' diffusion process of activation introduced by Heilbrunn and Wiercynski (1947). In any case, the final step in activation is an increase in concentration of calcium in the myofilament space of the cell.

The extensive distribution of the internal membrane system within the cell, and the fact that at least part of this system can accumulate calcium, raise the possibility that activation and relaxation *in vivo* involves intracellular translocation of calcium rather than movement across the cell membrane. Direct evidence comes from the studies of Winegrad (1965a, b, 1968, 1970). By exchanging some of the intracellular Ca^{++} with Ca^{45}, and using autoradiographic techniques, he was able to demonstrate the location of Ca^{45} in a resting and in an active muscle. For example, Ca^{45} was found to be

located mainly in (a) the terminal cisternae in a resting muscle, (b) the space between the myofilaments at the peak of a tetanus, and (c) in the A-band between the myofilaments (presumably in the intermediate cisternae and in the longitudinal tubules) in a declining tetanus. The fast exchange between Ca^{++} in the terminal cisternae and Ca^{45} in the bathing solution again demonstrated the accessibility of the terminal cisternae from the extracellular space. More recently, Connolly, Gough and Winegrad (1971) studied the effect of tetanus on the characteristics of the isometric twitch, and found maximal potentiation did not occur until about 10 to 12 seconds after the tetanus at 20°C. The shape of the isometric twitch as characterized by peak tension, maximum rate of tension development and contraction time, could be explained if two processes with half-times of about 2 seconds and 2 to 3 minutes occurred after a tetanus. The faster process also had a time course similar to that of the post-tetanic calcium movement in the sarcoplasmic reticulum. The relaxation phase of the twitch and the slow process were, however, not studied to any great detail. It seems certain that the characteristics of a twitch are governed mainly by the calcium distribution within the muscle cell, although a correlation between them is difficult. Studies of muscle activation along these lines are, however, useful to obtain indirect information on the underlying processes.

There are a number of other factors which have a direct or indirect influence on the characteristics of a twitch. Temperature variation is probably the most significant environmental factor

which causes changes in the rates of biological reactions. This is especially true among poikilothermous animals. The effect of temperature variation on biochemical and mechanical reactions is complex. It would be of value if direct experimental measurements of temperature could be compared with the effects of parameter changes in a model set up to represent the activation and contraction processes. Hill (1951c) reported the peak twitch tension in frog muscle was decreased when temperature was raised in the range 0 to 20°C while tetanus was somewhat increased. The fact that the maximum tension in a twitch was considerably less than in a tetanus had been attributed to oncoming relaxation allowing insufficient time for internal shortening to be completed. This would require that the temperature coefficient of the velocity of shortening would be substantially less than that of the decay of activity. Earlier, Hartree and Hill (1921) also found the temperature coefficient of the maximum rate of fall of tension in a twitch was about 1.5 times that of the maximum rate of rise of tension. In the neuromuscular system, however, the effects of temperature change are usually manifested in the rate change of some mechanical processes.

Hutchinson, Koles and Smith (1970) have reported a linear relationship between conduction velocity and temperature in single myelinated nerve fibers in *Xenopus laevis* (the African toad). Similar studies on the speed of response of muscle as characterized by the time course of twitches due to temperature variation have been scarce. Close (1965) and Close and Hoh (1968a) suggested that the differences in isometric twitch contraction times of different

muscles (fast and slow) might be due largely to differences in the twitch/tetanus ratio or peak twitch tension. A decrease in temperature decreased the intrinsic speed of shortening and the rate of removal of activator in all these muscles, thereby causing an inversely proportional increase in contraction time with little or no change in peak tension. In addition, it also increased peak tension of fast muscles in much the same way that repetitive stimulation increased it, by increasing the degree of activation of individual muscle fibers with little or no change in the time course of the response. Since the processes of activation, contraction and relaxation primarily involve the movement and distribution of intracellular Ca^{++}, it is important to relate them to the mechanical response of the muscle, and to temperature.

The characteristics of an isometric twitch can be studied using a model or by experimental investigation. The method of Julian (1969) for an activation-contraction model is extended in Chapters 2 and 3. The activation mechanism is extended to model repetitive stimulation and to include a threshold for contraction. Chapter 4 shows the effect of parameter changes of the model on the characteristics of the isometric twitch and the tetanus, and the rationale of changing any parameter is discussed. In later chapters, some effects of tetanus and temperature on the isometric twitch of the sartorius muscle of the bullfrog are demonstrated. Results obtained from time domain and frequency domain analyses are compared. The time course of the mechanical response is related to the speed of response of the contractile component and to rates of reactions of other processes.

CHAPTER 2

DESCRIPTION AND ASSUMPTIONS OF THE MODEL.

The components of the model and the major events in an activation-contraction cycle are shown in Figure 2.1.

2.1 *Force Generator.*

The basic units of the force generator are essentially those proposed by Huxley (1957) and consist of cross-bridges on the myosin filament and specific sites on the actin filament. A cross-bridge on the myosin filament is able to oscillate about its equilibrium position and to hook up with specific sites on the actin filament. Cross-bridges can only be made on one side of the equilibrium position. This results in net generation of force in one direction and a pulling action on the actin filament. Huxley (1957, p. 284) described the rate of change of interacting cross-bridges by a first-order equation:

$$\frac{\partial n}{\partial t} = (1 - n)f - ng \qquad (2.1)$$

where f and g are position-dependent rate constants of making and breaking of cross-bridges respectively, and n the proportion of interacting cross-bridges. Figure 2.2 is redrawn from Huxley (1957, p. 282) and shows the magnitude of f and g as a function of x, the distance of the thin filament site from the equilibrium position of the myosin cross-bridge; h is the positive distance

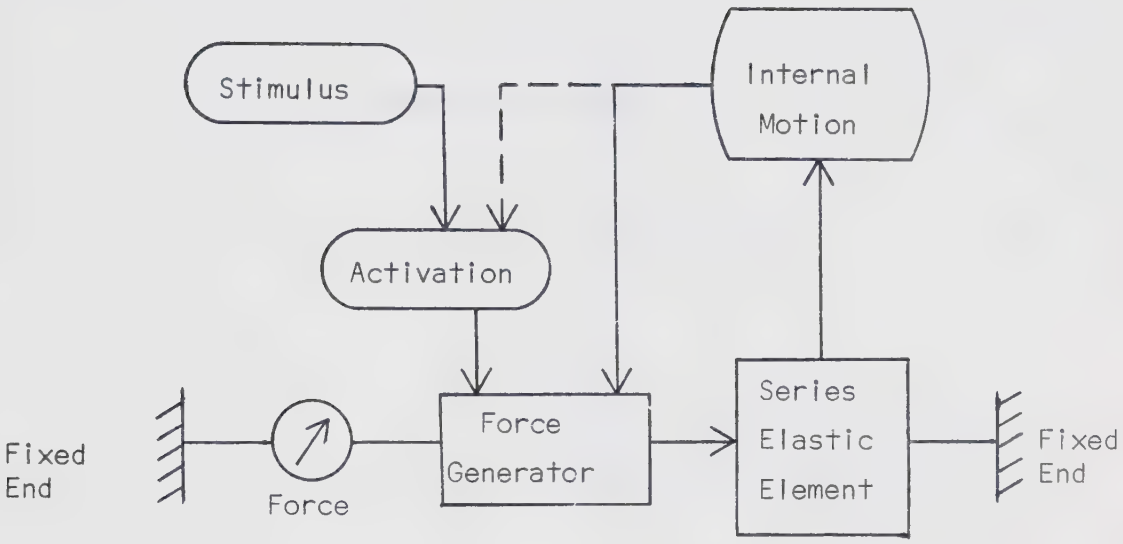

Figure 2.1 A block diagram of the model. Arrows indicate direction in which events are assumed to flow. The dashed line indicates possible motion feedback path. The circle labelled "force" shows the position of the force transducer.

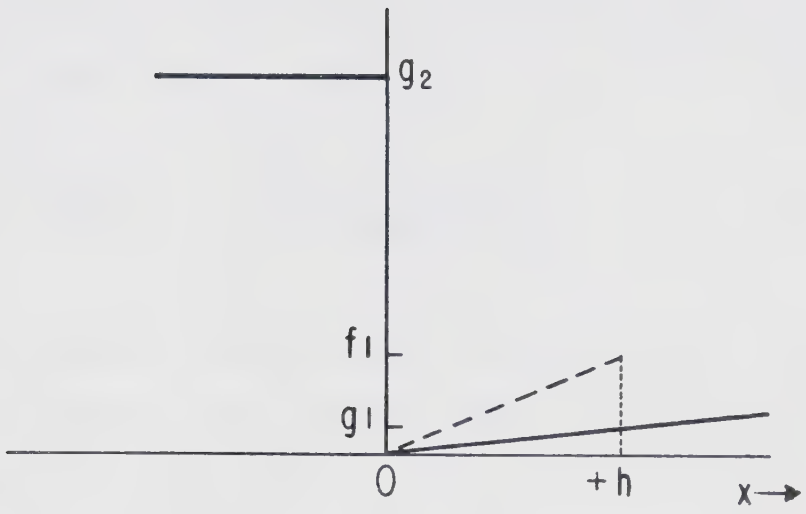

Figure 2.2 Dependence of f and g on x (from Huxley, 1957). Dashes for f and continuous line for g. f_1 and g_1 are respective values of f and g at $x = h$. g_2 is value of g when $x < 0$.

away from the equilibrium position beyond which a cross-bridge may not form a new interaction with a thin filament site and is assumed to have a value of 100 Å (Podolsky, Nolan and Zaveler, 1969). In the resting state, this interaction is prevented by some inhibition mechanisms.

Solving equation (2.1) with the constraint that the contractile unit is held isometric, gives:

$$n(u) = \frac{f}{f + g} - \frac{f - (f + g)n_o}{(f + g)\exp[(f + g)t]} \tag{2.2}$$

where u is the distance x normalized with respect to h; n_o is the initial n value at any x. As t becomes large, $n = f/(f + g)$, the isometric steady-state value for n.

The force, F, produced by an interacting cross-bridge at a distance x away from the equilibrium position is assumed to be kx, where k is a stiffness coefficient. The force produced by all the interacting cross-bridges is then:

$$F = \int_{-\infty}^{\infty} knxdx \tag{2.3}$$

The infinite upper and lower limits on the integral can be taken to mean that the integration is extended from the zero position until limits are reached at which the value for the integral no longer changes appreciably.

Reducing equation (2.3) to a normalized form suitable for the isometric steady-state by substituting $n = f/(f + g)$ gives:

$$\frac{F}{F_0} = \frac{f}{f+g}\frac{k}{k_0}\int_0^1 udu \qquad (2.4)$$

where F_0 is the maximum isometric force, and k_0 is a normalizing factor for the stiffness coefficient. Since $F/F_0 = 1$ in the isometric steady-state, $k/k_0 = 2(f+g)/f$. Equation (2.3) can now be written as:

$$P = \frac{2(f+g)}{f}\int_{-\infty}^{\infty} nudu \qquad (2.5)$$

where $P = F/F_0$. The equivalent, instantaneous stiffness of the force generator is given by:

$$k_g = \frac{2(f+g)}{f}\int_{-\infty}^{\infty} ndu \qquad (2.6)$$

2.2 *Activation.*

(i) Isometric Twitch.

An activation mechanism is necessary when non-steady-state processes, such as the time course of tension change in an isometric twitch is to be calculated. Following Julian (1969), the rate constant f which governs the rate at which cross-bridges interact is assumed to depend on the binding of calcium. Huxley's (1957) constraints on f as shown in Figure 2.2 are that $f = 0$ when $u < 0$ or $u > 1$. The value of f is weighed by an activation factor $\gamma(t)$ such that:

$$f = [\gamma(t)\ f_1]u \qquad (2.7)$$

$\gamma(t)$ varies between 0 and 1.

Experimental studies using the frog sartorius muscle (Jewell and Wilkie, 1958, 1960; Close, 1962) suggest that in an isometric twitch produced by a single stimulus, the active state rises to a peak in about 30 to 60 msec and decreases to a very low level by about 1.2 sec at 0°C. Jewell and Wilkie (1958) also recorded a twitch/tetanus ratio of up to .92, indicating that activation might be complete at this temperature. For these reasons, Julian (1969) assumed that in a twitch following a stimulus, γ rose from zero to a maximum 40 msec later, and then decayed back to very near zero 1.2 sec after the stimulus.

The time course of activation can be represented by two consecutive first-order reactions:

$$C \xrightarrow{\alpha} A \xrightarrow{\beta} E$$

where C = precursor;

A = activator;

E = end-product;

α = rate constant of binding of calcium by the myofilaments;

β = rate constant of release of calcium by the myofilaments and re-uptake by the sarcoplasmic reticulum.

C is assumed to be the amount of calcium released by a stimulus and E is a form of Ca^{++} which is ready for re-uptake into the sarcoplasmic reticulum. Reverse reactions are not allowed. Activation can only take effect when A is present.

The rate equations for these reactions are:

$$\frac{d[C]}{dt} = -\alpha[C] \tag{2.8}$$

$$\frac{d[A]}{dt} = \alpha[C] - \beta[A] \tag{2.9}$$

$$\frac{d[E]}{dt} = \beta[A] \tag{2.10}$$

where $[C]$ is the concentration of C.

Assuming that at $t = 0$, $[C] = C_0$, $[A] = [E] = 0$, equations (2.9) and (2.10) can be integrated to give the concentration of A:

$$[A] = \frac{C_0\ \alpha}{\beta - \alpha}\ [\ \exp(-\alpha t) - \exp(-\beta t)\ ] \tag{2.11}$$

$[A]$, the concentration of the activator, is assumed to be the amount of calcium bound to the myofilaments. The following type of function is selected to represent γ:

$$\gamma = \frac{[A]}{[A]_p} = \frac{1}{[A]_p}\ \frac{C_0\alpha}{\beta - \alpha}\ [\ \exp(-\alpha t) - \exp(-\beta t)\ ] \tag{2.12}$$

where $[A]_p$ = amount of calcium bound at peak of activation;

C_0 = amount of calcium released by one stimulus;

t = time in seconds.

Julian (1969) assumed that C_0 was the total calcium in 1 gm of muscle and it was given a value of 2 μmoles (Gilbert and Fenn, 1957). Using a value of $[A]_p$ = .15 μmole (Weber, 1966) and 40 msec for the time-to-peak activation, the rate constants α and β could be calculated from equation (2.12) and its first time derivative. In the present

model it is assumed that C_0 is the amount of calcium released by a single stimulus, instead of the total calcium in 1 gm of muscle. α also has a numerical value greater than β. These assumptions are more realistic because only a fraction of the total calcium is used in each twitch and binding of calcium by the myofilaments is a faster process (Huxley and Simmons, 1971). To keep the time course and magnitude of γ identical, the values of α and β were switched and C_0 was set to a fraction equal to .192/2 of the total calcium. The standard values for the variables governing the activation factor are thus $[A]_p$ = .15 μmole, C_0 = .192 μmole, α = 65/sec, β = 6/sec. The time course of the activation factor calculated using these values is shown in Figure 2.3.

(ii) Repetitive Stimulation.

To account for the time course of the activation factor in a fused tetanus, an arbitrary function can be written so that it increases from zero to one in a way similar to that given by equation (2.12) but remains at one thereafter (Julian, 1969). If the frequency of stimulation is low so that an unfused tetanus results, the time course of the activation factor following each stimulus must be calculated. If A_0 is the amount of residual activator substance as a result of previous activity, then solving equations (2.9) and (2.10) gives:

$$\gamma = \frac{1}{[A]_p} \frac{C_0 N \alpha}{\beta - \alpha} [\, \exp(-\alpha t) - \exp(-\beta t) \,] + \frac{A_0}{[A]_p} \exp(-\beta t) \quad (2.13)$$

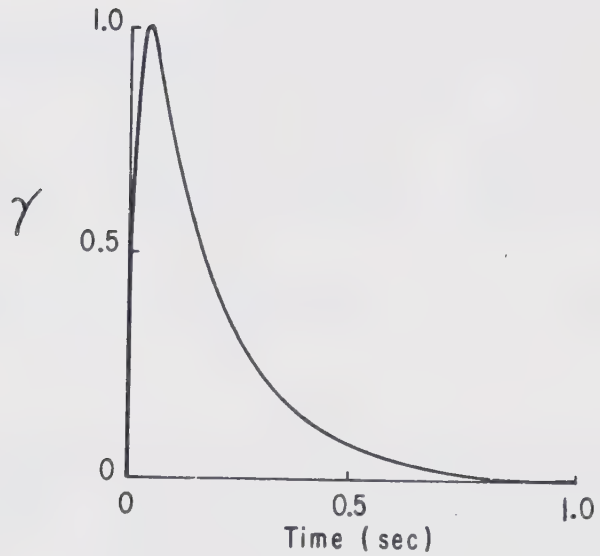

Figure 2.3 Postulated time course of the activation factor, γ, for isometric twitch (equation (2.12)).

where $C_{0,N}$ is the amount of calcium released by the N^{th} stimulus.

If S_N = amount stored before the N^{th} stimulus,

$$C_{0,N} = \left(\frac{.192}{S_1} \right) S_N$$

$$S_N = S_{N-1} - C_{0,N-1}$$

Solving these equations gives:

$$S_N = S_{N-1} \left(1 - \frac{.192}{S_1} \right) = S_1 \left(1 - \frac{.192}{S_1} \right)^{N-1}$$

and:
$$C_{0,N} = .192 \left(1 - \frac{.192}{S_1} \right)^{N-1} \qquad (2.14)$$

From equation (2.14), it is obvious that $C_{0,N}$ is greatest in the first stimulus. Any subsequent values are decreased as the number of stimuli is increased. The amount of calcium that remains unbound immediately before a stimulus (other than the first) is small and is neglected in the computation. This is because any free Ca^{++} is readily bound (with a rate α = 65/sec), as can be seen from equation (2.8).

The inclusion of the factor A_0 in equation (2.13) enables γ to have values greater than 1 under certain circumstances. However, the tension produced can only increase to a maximum of about 1.2 for reasons which will be given later. In fact $\frac{A_0}{[A]_p}$ has a value equal to γ immediately before the next stimulus is delivered. Its magnitude depends on the previous value of A_0 and the time lapse since the

previous stimulus.

(iii) Threshold.

The necessity to include a threshold for developing tension follows from the fact that in slow muscle fibers, a single stimulus does not elicit a twitch; considerable tension can only be generated by repetitive stimulation (Kuffler, Laporte and Ransmeier, 1947; Kuffler and Vaughan-Williams, 1953). Studies on the mechanical threshold of slow muscle are very few. Although a strict comparison is impossible, it is important to have an approximate value of Ca^{++} concentration ($[Ca^{++}]$) for the threshold. Weber and Winicur (1961) found a threshold $[Ca^{++}]$ of about .2 µmole for a 'natural' actomyosin preparation and Portzehl, Caldwell and Ruegg (1964) reported a value of .3 to 1.5 µmole for muscle fibers of the crab.

Julian (1971) studied the effect of calcium on the force-velocity relation of briefly glycerinated frog muscle fibers. He observed the contractions induced and the steady force developed at about 4°C by lowering the pCa in the relaxing solutions to various levels determined by the ratio of calcium and EGTA added. The relationship between steady force generated and pCa was S-shaped and very steep. Contractions were never observed above pCa 7. The data for his Force-pCa curve (Julian, 1971, p. 130) are replotted in Figure 2.4 with $[Ca^{++}]$ instead of pCa. Below a $[Ca^{++}]$ of .12 µmole, no force is produced; and the steady force rises from zero to half its maximum value with a change of $[Ca^{++}]$ of .05 µmole.

To find the value of γ which corresponds to $[Ca^{++}]$ = .12 µmole,

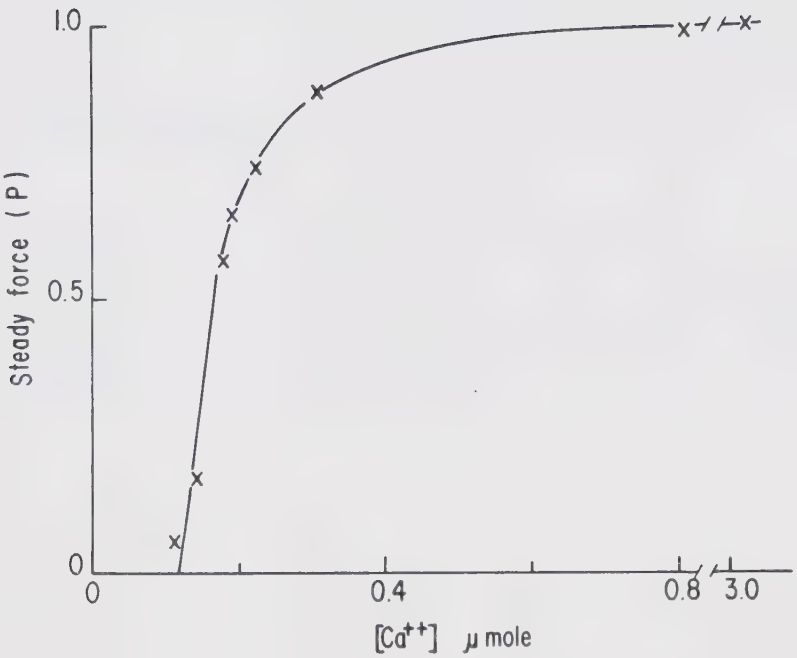

Figure 2.4 Tension - $[Ca^{++}]$ curve for determination of threshold $[Ca^{++}]$ for contraction. (Data from Julian, 1971).

the value of n in equation (2.2) is considered. At the isometric steady-state, $n = f_1/(f_1 + g_1) = .8125$, using values of $f_1 = 81.25$/sec and $g_1 = 18.75$/sec (Julian, 1969). The steady force P is related to γ as follows:

Suppose $f = 81.25\ \gamma$ (so that when $\gamma = 1$, $f = f_1 = 81.25$)

$$\text{Then } \gamma = \frac{f}{81.25} \tag{2.15}$$

In Figure 4, when $P = \frac{1}{2}$, $n = f/(f + g_1) \simeq \frac{1}{2}$ or $f = g_1$, assuming the steady-state value of n at any P depends only on f. Substituting this value for f into equation (2.15):

$$\gamma_{\frac{1}{2}P} = \frac{18.75}{81.25} \tag{2.16}$$

where $\gamma_{\frac{1}{2}P}$ is the value of γ at $P = \frac{1}{2}$.

This value of γ is required for the tension to increase from zero to half its maximum, with a corresponding change of $[Ca^{++}]$ of .05 μmole, so that a threshold at $[Ca^{++}] = .12$ μmole in unit of γ equals:

$$\gamma_{\text{threshold}} = \frac{.12}{.05}\ \gamma_{\frac{1}{2}P} = .554 \tag{2.17}$$

This amount can be subtracted from the equation for the activation factor, given by equations (2.12) or (2.13) if a threshold phenomenon in contraction is to be modelled.

2.3 *Series Elastic Element.*

The presence of series elastic element in a muscle determines to some extent the time course of tension change manifested externally. Jewell and Wilkie (1958) determined the load-extension characteristic of frog sartorius muscle at 0°C. Their non-linear load-extension curve could be divided into two regions, with values of stiffness ranging from about 0.3 to 0.6. These are normalized values with respect to the maximum isometric tension and the maximum extension per half-sarcomere in unit of h (Section 2.1). Since the effect of changes of parameters in the model is studied, a standard value of 0.5 is given to the stiffness of the series elastic element. This value gave a twitch nearly identical to that computed by Julian (1969).

2.4 *Activation-Contraction Coupling and Internal Shortening.*

Activation-contraction coupling is an essential part in muscle function, but does not enter explicitly into the calculations. It is assumed that the activation process begins from the binding of calcium by the myofilaments, and can be separated from any previous events following a stimulus. Besides the threshold phenomenon, another difference between fast (twitch) and slow fibers in frogs is the presence of all-or-none action potentials in the former. The assumptions in the model are therefore less true for slow fibers. An explicit model for activation and activation-contraction coupling for these fibers might have to be included in future work.

Although the contractile unit is kept isometric, internal

shortening is still possible because of the series elastic element. The way in which this affects the activation is an inherent property of the model itself.

CHAPTER 3

METHODS FOR COMPUTATIONS.

The computation method for the standard isometric twitch is described in this chapter. Except for the modifications given in Chapter 2, the method is basically similar for tetanus (repetitive stimulation) or when a threshold is included. A Digital Equipment Corporation LAB-8 computer was programmed to do the calculations.

In the mathematical formulation of the sliding filament model (Huxley, 1957), the rate constants f and g for positive u are proportional to f_1 and g_1, the values for f and g at $u = 1$. For negative u, $g = g_2$ and is independent of u (Figure 2.2). However, only values for the ratio of rate constants were given in that model. To compute the time course of tension change, absolute values for the rate constants are required. From Julian's paper (1969), these are: $f_1 = 81.25$/sec, $g_1 = 18.75$/sec and $g_2 = 391.9$/sec. These values were chosen to satisfy the ratios of the rate constants and to give a reasonable value of the maximum unloaded shortening velocity given by the equation $V_m = 4mh\ (f_1 + g_1)$, where m is the number of sarcomeres (Huxley, 1957, p. 288). Using an approximate value $m = 10^4$, and values already given for h, f_1 and g_1 yields $V_m = 4$ cm/sec.

The generator's force and equivalent stiffness given by equations (2.5) and (2.6) respectively can be calculated when n is known as a function of u. Using a small time increment Δt to approximate a change in the continuous time function for γ, f as a function of both γ and u, and g as a function of u, equation (2.2) becomes:

$$n(u) = \frac{f(\gamma,u)}{f(\gamma,u) + g(u)}$$

$$- \frac{f(\gamma,u) - [f(\gamma,u) + g(u)]\, n_0}{[f(\gamma,u) + g(u)]\, \exp\{[f(\gamma,u) + g(u)]\Delta t\}} \qquad (3.1)$$

Equation (3.1) can be written to apply in three distinct regions over which u varies (see Figure 2.2):

(1) $-\infty < u < 0$: $f = 0$, $g = 391.9$

$$n = n_0/\exp(391.9t) \qquad (3.2)$$

(2) $0 \leq u \leq 1$: $f = \gamma(t)\ [81.25u]$, $g = 18.75u$

$$n(u) = \frac{\gamma(t)(81.25)}{\gamma(t)(81.25) + 18.75}$$

$$- \frac{\gamma(t)(81.25) - [\gamma(t)(81.25) + 18.75]\, n_0}{[\gamma(t)(81.25) + 18.75]\, \exp\{u[\gamma(t)(81.25) + 18.75]t\}} \qquad (3.3)$$

(3) $1 < u < \infty$: $f = 0$, $g = 18.75u$

$$n(u) = n_0/\exp[u(18.75)t] \qquad (3.4)$$

where t is the total time increment.

Equation (2.5) can be written as:

$$P_g = \frac{2(f_1 + g_1)}{f_1} \left[\int_A^0 nudu + \int_0^1 nudu + \int_1^B nudu\right] \qquad (3.5)$$

where P_g = generator's force; A and B are as yet undefined limits. A simple numerical integration method is used to compute the above integrals. Initially a value for γ is calculated at time = $\frac{1}{2}\Delta t$. Any subsequent increment is equal to a full Δt. In this way the intermediate value is used for any interval and is assumed to approximate the average for that interval. The total length change, L, of the force generator and that of the series elastic element are equal because they are connected in series. This length is zero at $t = 0$. The computer then checks the particular region of u over which n varies and then proceeds to sum up all n in that region. In the regions of positive and negative u, n is computed until a value of $n = 0$ is encountered. This occurs when $n < .0005$ because of the word length (12 bits) for fixed point numbers in the range ±1 in this computer. The limits A and B in equation (3.5) are then set by the extent to which n is shifted into regions of negative and positive u respectively. The generator's force can then be computed by numerical integration. The same procedure is used to compute the generator's equivalent stiffness.

At the end of a time step Δt, the generator exerts the force P_g and has an equivalent stiffness k_g. Equilibrium is established when the net force in the generator is equal to the force in the series elastic element, and is given by the following equation:

$$P_g - k_g L = kL \tag{3.7}$$

where k is the stiffness of the series elastic element and L is the

total length change from zero time. Rearranging equation (3.7) gives:

$$L = \frac{P_g}{k_g + k} \tag{3.8}$$

The force measured externally is then equal to:

$$P = kL \tag{3.9}$$

Addition of the stiffness of the force generator and that of the series elastic element results from the development by the generator in the active state of a stiffness which impedes both shortening and lengthening of itself.

At all steps, n, t, du, L, P_g and γ are stored in the computer. For calculating the force and length change at a later time, the amount of shortening or lengthening is considered when calculating the region for u, and the effect of n in all previous t is included.

In equations (2.12) and (3.1) to (3.5), values of Δt = 5 msec and du = .025 are used. A smaller Δt would give a more accurate result, but the amount of computer time needed becomes too large. Halving these values does not change results appreciably ($< 5\%$). The required data are printed out using a teletype machine.

CHAPTER 4

RESULTS OF COMPUTATIONS.

4.1 *Abbreviations and Definitions.*

The following abbreviations are used to describe the characteristics of an isometric twitch (Close, 1972):

P_t : tension at the peak of an isometric twitch;

dP/dt : maximum rate of tension development;

T_c : contraction time, i.e., the time from onset to peak of an isometric twitch;

$T_{\frac{1}{2}r}$: half-relaxation time, i.e., the time for tension to fall from P_t to $\frac{1}{2}P_t$ during the relaxation phase of an isometric twitch;

τ : the time constant for the decay of tension during the exponential phase of relaxation at the end of an isometric twitch.

4.2 *Isometric Twitches.*

(i) Parameter Changes of The Activation Factor.

Figure 4.1 shows isometric twitches computed for different β, the rate of release of calcium from the myofilaments. The standard twitch (labelled "β" in Figure 4.1) is computed using the standard values for the parameters given in Chapters 2 and 3, and activation is calculated to reach a maximum 40 msec following a single stimulus

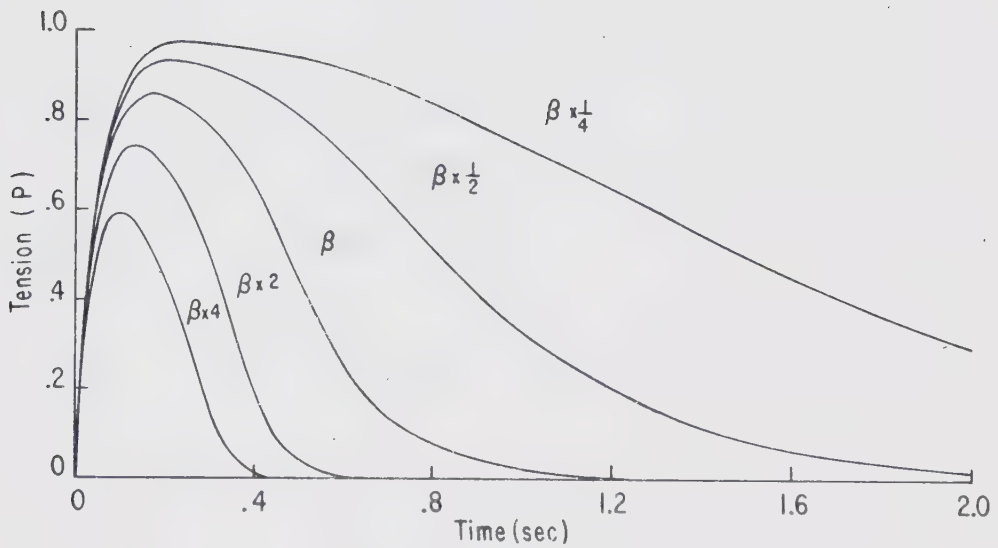

Figure 4.1 Computed isometric twitches for different β. The multiplicative factor is listed on the right of each curve. The curve labelled "β" is the 'standard' twitch for 0°C computed using 'standard' values for the activation factor. A tension of 1.0 indicates maximum isometric tension.

at a temperature of 0°C. It can be seen that changes in β have the greatest effect on the relaxation phase of the twitch, although P_t is also affected considerably. Figure 4.2 shows the semi-logarithmic plots of the fall of tension during relaxation of the different twitches shown in Figure 4.1. In all cases the tension record shows a final exponential fall. Jewell and Wilkie (1960) observed this exponential fall of tension in relaxing muscle, and gave evidence that the rate constant of tension fall appeared to be characteristic of a particular muscle under given conditions. The rate constant ($1/\tau$) of fall of tension from 60% to 5% of P_t of the standard twitch is about 5.75/sec. For twitches computed with different β, these rates are normalized to the standard rate and are listed in Figure 4.2. The relaxation rate is approximately doubled with doubling of β except for the highest rate (β × 4).

The effects of other activation parameters have also been computed. These include C_0, the amount of calcium released by one stimulus and α, the rate of binding of calcium to the myofilaments. A fourth one, designated SR, stands for volume of sarcoplasmic reticulum, assumes that both C_0 and β are changed by the same factor. It is assumed here that the existence of a smaller volume of sarcoplasmic reticulum will cause a smaller amount of calcium to be released, from both the sarcoplasmic reticulum itself and the bound state of the myofilaments. In the computation, both C_0 and β are changed arbitrarily by the same factor.

To compare the effects of parameters changes of the activation factor on the characteristics of isometric twitch, the para-

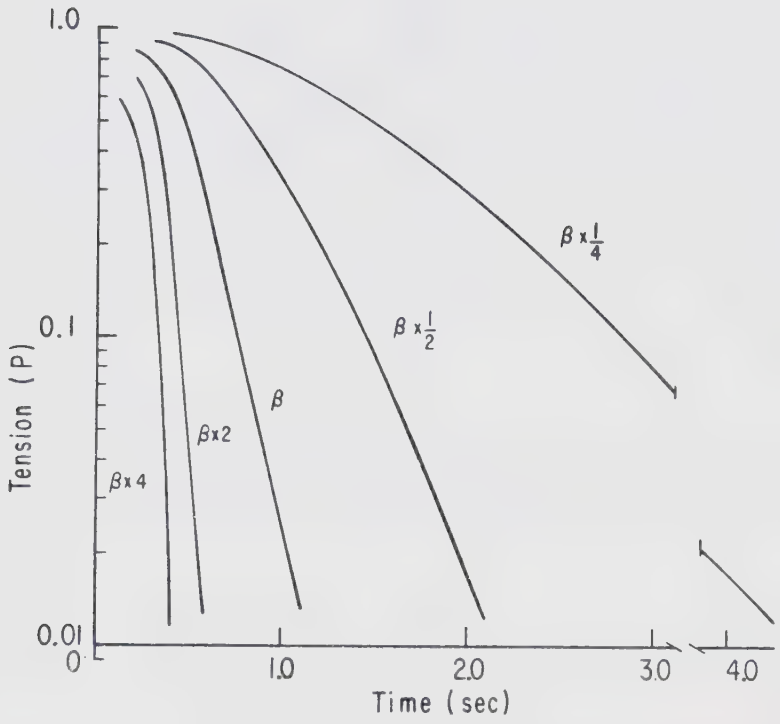

Figure 4.2 Semi-logarithmic plots of the fall of tension during relaxation in the computed twitches shown in Figure 4.1. The relative rate constants of fall of tension from 60% to 5% of the respective maxima are: 3.2 ($\beta \times 4$), 2.2 ($\beta \times 2$), 1 (β), .45 ($\beta \times \frac{1}{2}$), .22 ($\beta \times \frac{1}{4}$).

meters and the characteristics relative to their 'standard' values are plotted on a log-log scale as shown in Figure 4.3. Parameters of the activation factor changed by a factor of two and four are shown. Several results follow immediately from Figure 4.3:

(1) The largest change observed is the change in $T_{\frac{1}{2}r}$ for $\beta < 1$, where a direct proportionality is observed. A proportionality was also observed for the rate constants of tension fall with different β, as previously mentioned. The other characteristics appear to depend on several parameters to a varying extent.

(2) The time course of the contraction phase as defined by dP/dt and T_c is more sensitive to changes in C_0 or SR.

(3) The time course of relaxation as defined by $T_{\frac{1}{2}r}$ is most sensitive to change in β.

(4) α seems to have the least effect on the characteristics of the isometric twitch.

(ii) Parameter Changes of the Original Sliding Filament Model.

Figure 4.4 shows results obtained by changing independently the parameters in the original sliding filament model (Huxley, 1957). These are the boundary rate constants for the making and breaking of cross-bridges. Also included is the stiffness of the series elastic element which does not exist in the original model. Its effect will be considered in more detail in the next section. Several obvious

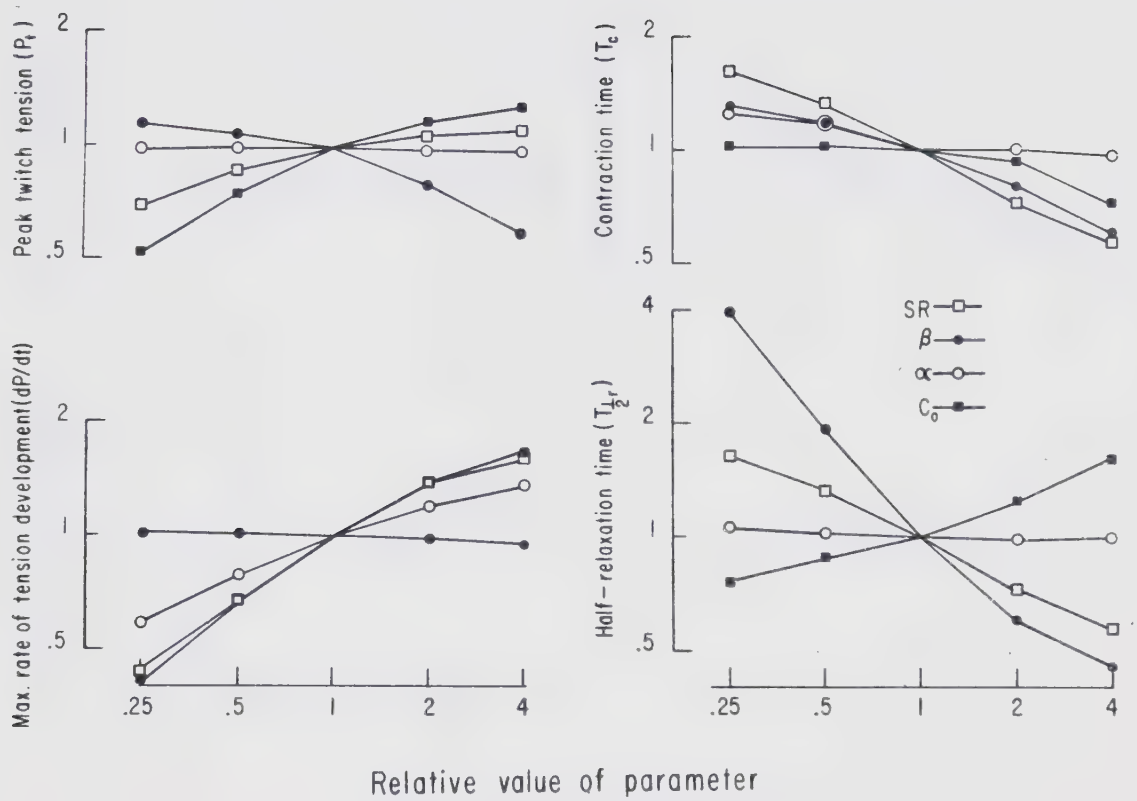

Figure 4.3 Effects of changing parameters in the activation factor on the characteristics of the isometric twitch. All values have been normalized to their respective 'standard' values.

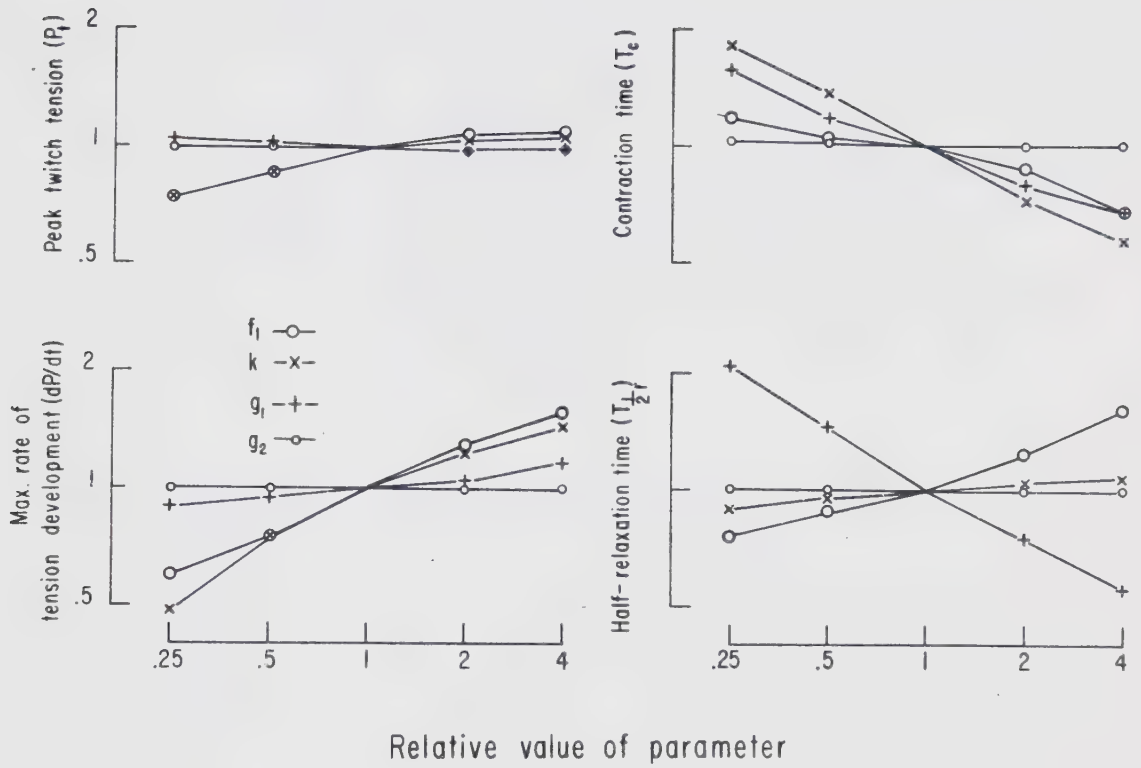

Figure 4.4 Effects of changing parameters in the original sliding filament model and k (series stiffness) on the characteristics of the isometric twitch. All values have been normalized to the 'standard' values.

results are outlined below:

(1) Similar to the parameters of the activation factor, the twitch characteristics depend on several parameters and are affected less than proportionally by changes in any one of them;

(2) The peak twitch tension is rather insensitive to any one parameter;

(3) The contraction phase is more sensitive to f_1 or k.

(4) The relaxation phase is most sensitive to g_1.

(5) g_2 has negligible effect on the characteristics of an isometric twitch.

(iii) Effect of the Series Elastic Element.

Figure 4.5 shows the isometric twitches computed for different k. In the model k has the greatest effect on the contraction phase (see also Figure 4.4). Jewell and Wilkie (1960) reported that k had very little effect on the exponential relaxation phase. Hill (1951a) showed that an added series compliance to an 'isometric' twitch of frog sartorius affected the time course of tension development. To compare, the series stiffness k in the model was converted to absolute units of compliance. Added compliance was obtained after subtracting the compliance of the standard twitch which was assumed to have zero added compliance. Its effect on the characteristics P_t, dP/dt and T_c is shown in Figure 4.6. Data from Hill, 1951a, Figure 1) on the effect of an added compliance on the twitch are also plotted in Figure 4.6. The standard value of .5 for

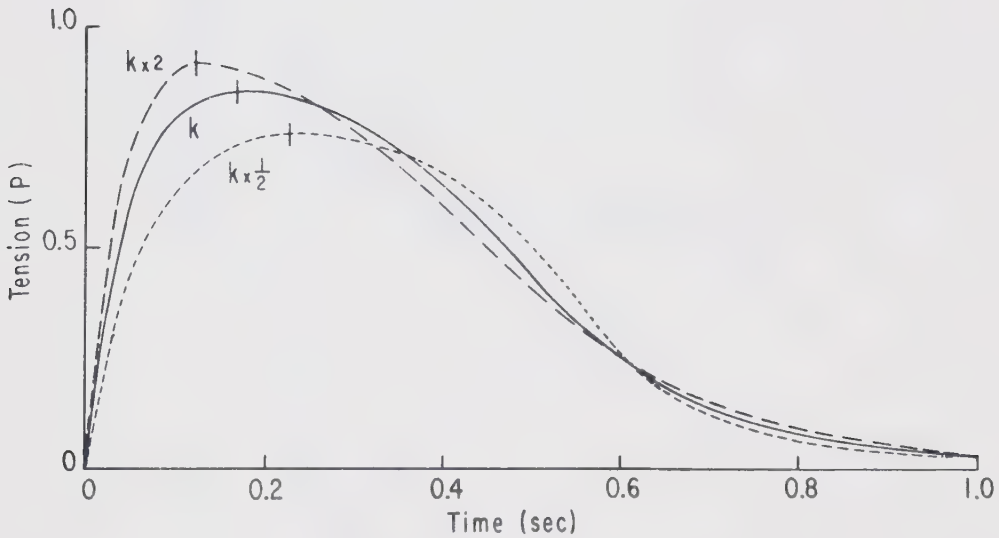

Figure 4.5 Computed isometric twitches for different k. The multiplicative factor is listed with each curve. The curve labelled "k" is the 'standard' twitch.

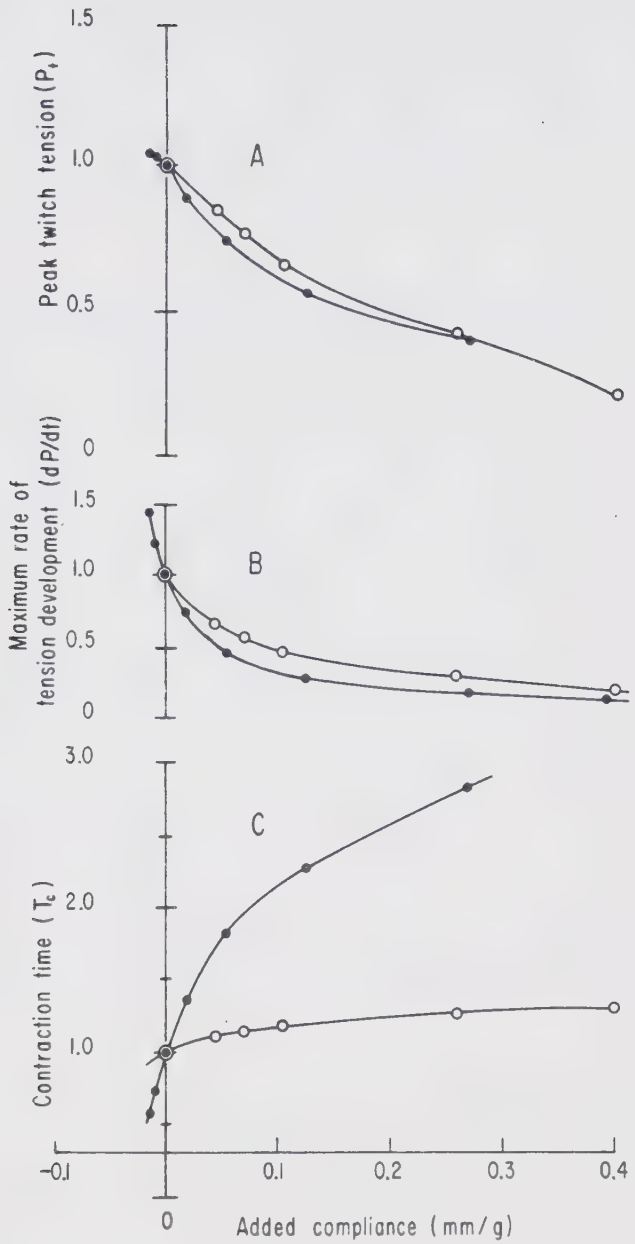

Figure 4.6 Effects of added compliance on the characteristics of the isometric twitch. O, experimental data from Hill (1951a); ●, data from model.

k is equivalent to a compliance of .018 mm/gm. Good agreement is found between the two sets of data for P_t and dP/dt. However, the series elastic element in the model produces greater changes in T_c than are found experimentally (Figure 4.6C).

4.3 *Tetanus (Repetitive Stimulation) and Threshold.*

Figure 4.7 shows the tension curves obtained by stimulating the 'standard' muscle at a rate of 10/sec, together with a muscle with $\frac{1}{2}SR$ as defined previously. It can be seen that the tetanic tension curves follow the same time course initially as the corresponding twitch up to .1 sec. At the end of each stimulus, A_0 and a new value of $C_{0,N}$ are computed. These new values are then used to compute the tension curve for the next stimulus. The fraction of interacting cross-bridges due to the previous stimulus is retained by the computer. As explained in Chapter 2, $C_{0,N}$, the amount of calcium released by one stimulus, becomes smaller with each additional stimulus.

Several differences are obvious between the two tension curves; the muscle with $\frac{1}{2}SR$ attains a smaller twitch tension, has a longer contraction time and a lower rate of tension development, but the tension during repetitive stimulation is better maintained.

As defined by Julian (1969), the largest value γ could attain in the 'standard' activation factor is 1, but with repetitive stimulation in the present model, γ can be several times greater. From equation (2.7), this means that f will also be increased several times. However, the generator's force can only reach a certain limit

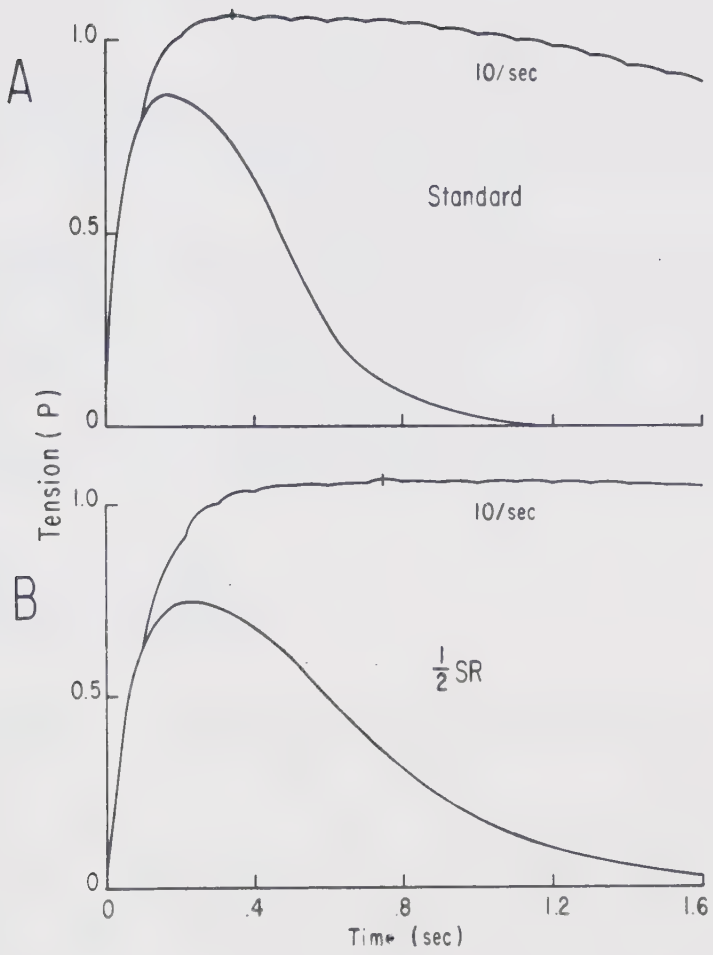

Figure 4.7 Computed isometric twitch and tetanus (10/sec) of A) 'standard' muscle and B) '$\frac{1}{2}SR$' muscle. The peaks of the tetani are also marked.

for the following reason. From equation (2.2), it can be seen that as f becomes very large, n approaches a limiting value of 1. Substituting $n = 1$ and $(f + g)/f = .8125$ into equation (2.5) and evaluating P_g using the limits of integration for isometric steady-state gives:

$$P_g = 2(.8125) \int_0^1 udu = 1.23 \qquad (4.1)$$

This is the maximum force which the generator can attain when there is no internal shortening. The force P measured externally is, however, always smaller than P_g, as is obvious from the following equation after substituting L from equation (3.8) into equation (3.9):

$$P = kL = k \frac{P_g}{k + k_g} < P_g \qquad (4.2)$$

It is also obvious that when k is very large (approaching a true isometric condition) $P \rightarrow P_g$.

Indirect experimental evidence on human muscle indicates that the active state cannot only be prolonged, but can also be intensified (Desmedt and Hainaut, 1968).

The activation factor is calculated for 0°C. At this low temperature the twitch/tetanus ratio is high and equal to .81 for the 'standard' muscle compared to a lower value of .70 for the '$\frac{1}{2}SR$'.

A threshold value of .554 for γ is derived in Chapter 2. If this is deducted from the activation factor for '$\frac{1}{2}SR$', no tension will be generated when only a single stimulus is delivered. This is

because $C_{0,1}$ is reduced by half in the calculation of γ in '$\frac{1}{2}SR$' and the maximum value for γ in this case is .5, which is smaller than .554. When this is encountered, γ is set equal to zero in the computation. However, in repetitive stimulation γ can exceed the threshold value of .554 and tension can then be generated. Figure 4.8 shows the tension curve of a 'standard' and a '$\frac{1}{2}SR$ plus threshold' muscle stimulated at a rate of 20/sec. The results are similar to that at 10/sec although at higher rates the effects are more prominent.

The muscle with '$\frac{1}{2}SR$ plus threshold' does not generate any tension until after the second stimulus. Comparing with the 'standard' muscle, it also reaches a smaller peak tension at a longer time, a smaller rate of tension development, a smaller fusion frequency and a low fatiguability.

4.4 *Frequency-Tension Characteristic.*

Muscle tension increased with increasing frequency of stimulation in a characteristic way (Cooper and Eccles, 1930; Matthews, 1959; Rack and Westbury, 1969). The frequency-tension curve has a sigmoid form. There is always a middle range within which tension rises steeply with each increment in frequency, but further increase in stimulation beyond that range has diminishing effects on the tension.

If the parametric modelling of activation for the three types, 'standard', '$\frac{1}{2}SR$', and '$\frac{1}{2}SR$ plus threshold', represents different patterns of activation in a muscle, or in different muscles,

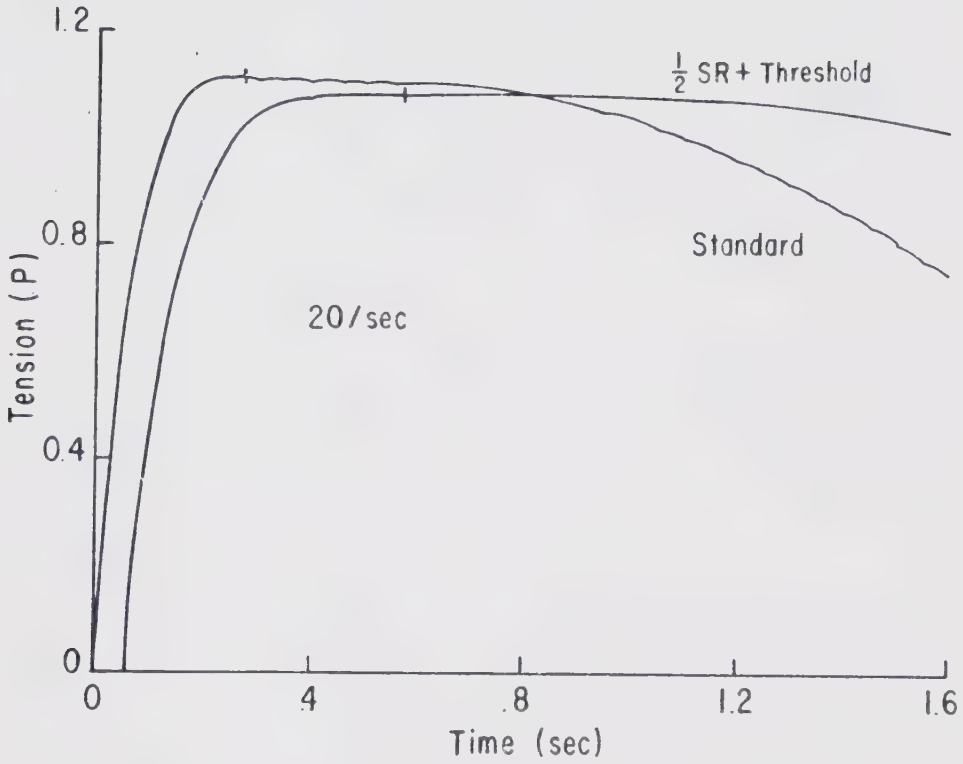

Figure 4.8 Computed tetanus (20/sec) of the 'standard' muscle and the '$\frac{1}{2}SR$ + threshold' muscle. The peaks of the tetani are also marked. Note tetanic tension in the '$\frac{1}{2}SR$ + threshold' muscle begins to develop only after the second stimulus.

their frequency-tension relations will reveal the characteristic pertinent to each type. At all frequencies of stimulation the tension usually reaches a peak and then declines, whether the tetanus is partially fused or completely fused. This is due to the residual A_0 and n from the previous stimulus and a proportionally smaller C_0 for each additional stimulus. For this reason, the portion of tension record containing the peak and between two stimuli is averaged with respect to time for the partially-fused or completely-fused tetanus, while in a twitch the time-average of the whole record is considered.

Figure 4.9 shows the frequency-tension relations for these three types of parametric modelling obtained in this way. The curve for the '$\frac{1}{2}SR$ plus threshold' has a sigmoid shape while the curves for the 'standard' and '$\frac{1}{2}SR$' rise very sharply.

In real muscles the sigmoid curve is always observed for the frequency-tension relationship. This is true in a slow twitch muscle such as the soleus muscle (Matthews, 1959; Rack and Westbury, 1969; Mannard and Stein, 1972), or in fast twitch muscle such as the gastrocnemius in cat (Kernell, 1966). The maximum rate of rise of the frequency-tension curve is greater in a fast muscle and Matthews (1959) reported by stretching the soleus muscle, the curve shifted towards the lower frequency end (the muscle had a lower fusion frequency) and had a greater maximum slope. In the present model the existence of the sigmoid shape is a result of the threshold.

Tension does not appear until frequency is just above 1/sec in the '$\frac{1}{2}SR$ plus threshold' muscle for the reason which is given earlier.

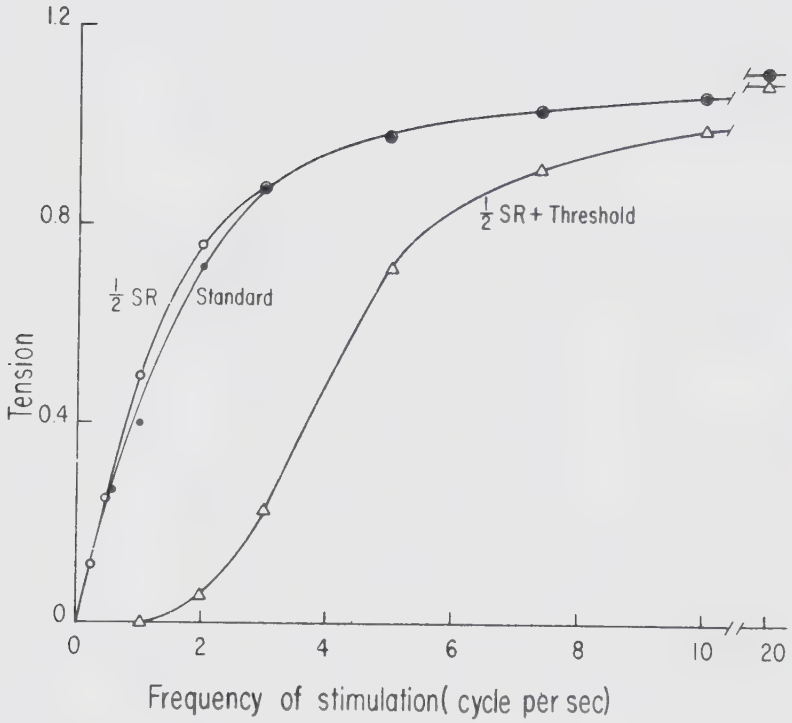

Figure 4.9 Frequency-tension curves of three muscles with different activation mechanisms: ●, 'standard'; ○, '$\frac{1}{2}SR$'; △, '$\frac{1}{2}SR$ + threshold'. Note sigmoid shape of curve for the '$\frac{1}{2}SR$ + threshold' muscle and absence of tension below 1 cycle/sec.

At low frequencies, the '$\frac{1}{2}SR$' muscle actually gives a greater average tension than the 'standard' muscle. This is because although C_0 in the '$\frac{1}{2}SR$' muscle is only half of that in the 'standard' muscle, the smaller β greatly prolongs the duration of activation and thus the time course of the relaxation phase. Therefore, summation of force occurs from one stimulus to the next. At high frequencies the two curves are almost identical; here the peak tension is approaching the limiting value (1.23) of the maximum possible tension in the model.

In the soleus muscle of the cat at 37°C, a stimulus frequency of 30/sec already gives a tension more than 90% of the maximum at the optimum length (Matthews, 1959; Rack and Westbury, 1969). In the model a corresponding frequency would be about 10/sec. This is mainly because activation is originally derived for 0°C, a temperature at which fusion frequency is low and activation reaches its peak even in a single twitch, and partly because the greatly increased γ tends to make n approach its maximum value of 1, thus giving a high tension even at a frequency of 10/sec.

4.5 *Fourier Transform of the Isometric Twitch.*

The direct Fourier transform is a tool that resolves a signal into its frequency components, and gives a frequency domain representation of the signal. Time domain representation specifies a function at each instant of time, whereas frequency domain representation specifies the relative amplitudes of the frequency components of the function.

In a nerve-muscle preparation, the action potentials can be considered as a series of unit impulses (French and Holden, 1971a; Knox, 1969) and as an input to the muscle which develops tension as its output. Since a motoneuron usually conveys information in the form of its impulse frequencies, frequency domain analysis applied to the neuromuscular system is of great value in revealing the signal-handling characteristic of a muscle (Partridge, 1966). Methods have been developed by French and Holden (1971a, b, c) for estimating the frequency response of a muscle using spectral analysis. Alternatively, if the nerve-muscle preparation were considered as a linear system, the twitch could be used as the impulse response of the system, and the Fourier transform of the twitch would give the frequency response (D'Azzo and Houpis, 1966).

A Fourier transform generates complex numbers which need two plots for their graphical representation as a function of frequency, e.g. plots of magnitude and of phase. Figure 4.10 shows the magnitude of the Fourier transform and the phase angle as a function of log frequency of the standard isometric twitch. The magnitude plot essentially gives a prediction for the responsiveness of the muscle in terms of the change in tension which should be produced for each impulse per second modulation in stimulus rate, if the latter were being modulated sinusoidally with the frequency given on the abscissa. The fitted curves in Figure 4.10 are the magnitude and phase which would be expected for a critically-damped, second-order system of the form:

$$G(s) = \frac{G_0}{s^2 + 2\zeta f_n s + f_n^2} \qquad (4.3)$$

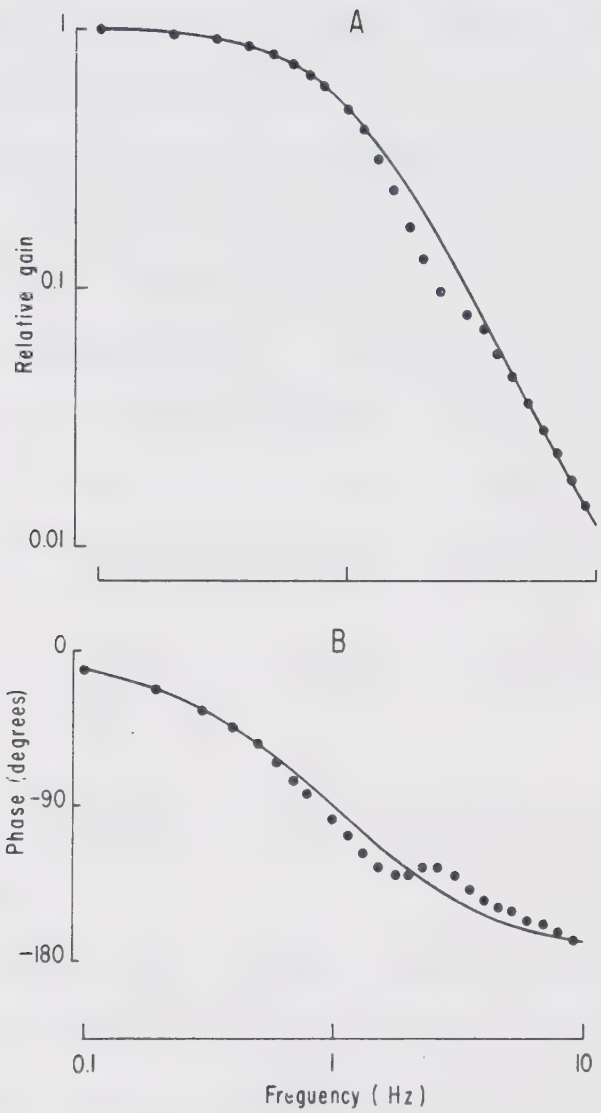

Figure 4.10 The magnitude (A) and phase (B) of the frequency response of the 'standard' muscle obtained by Fourier transformation of the isometric twitch. Further explanation in text.

where s is the Laplace transform variable; $G(s)$, the transfer function representing the frequency response; G_0, the zero frequency gain; f_n, the undamped natural frequency; and ζ, the damping ratio of the system. For critical damping, ζ is equal to 1.

A second-order system is completely defined by the three parameters G_0, f_n and ζ in its gain curve which is a plot of the magnitude of $G(s)$ against frequency (the continuous curve in Figure 4.10A).

The zero frequency gain is the magnitude of the direct Fourier transform when frequency approaches zero and determines the vertical position of the curve. It is equal to the area under the tension record of a twitch as can be seen from the following equation:

$$\lim_{f \to 0} F(f) = \lim_{f \to 0} \int_{-\infty}^{+\infty} f(t)\, e^{-2\pi f t}\, dt = \int_{-\infty}^{+\infty} f(t)dt \qquad (4.4)$$

where $f(t)$ is the tension curve (in the time domain) and $F(f)$ is the Fourier transform.

The undamped natural frequency is the frequency with which the system will oscillate if $\zeta = 0$ and determines the horizontal position of the gain curve. It is the frequency at which G_0 decreases to half its value in the gain curve for a critically-damped, second-order system. It is related to the speed of response of the system in the time domain. Two characteristics which determine the speed of tension change in an isometric twitch are the maximum rate of tension development and the rate of tension decay. The larger the f_n, the faster the system responds.

The damping ratio while specifying the shape of the gain

curve in the frequency domain, also determines the shape of the twitch in the time domain. A critically-damped system has $\zeta = 1$; any value smaller or greater than 1 indicates, respectively, underdamping or overdamping. In a second-order system given by equation (4.3), the impulse response (the inverse Fourier transform) for $\zeta > 1$ is highly skewed whereas for $\zeta < 1$ some overshoot (oscillation) is observed (Ogata, 1970).

If the frequency response of the standard muscle is represented as a critically-damped, second-order system, it has a natural frequency of about 1 Hz (Figure 4.10A). The steep fall-off of the Fourier transform at high frequencies effectively describes also the low-pass characteristic of the contractile unit in the model. In real muscles this characteristic has been reported in frequency response studies under isometric conditions (Partridge, 1965; Poppele and Terzuolo, 1968; Rosenthal, McKean, Roberts and Terzuolo, 1970; Mannard and Stein, 1972) and isotonic conditions (Partridge, 1966).

Figure 4.11 shows the Fourier transforms of isometric twitches computed by changing β, SR and k by a factor of two. The response of the standard muscle is included for comparison. The representation by a critically-damped, second-order system is also good in most of the Fourier transforms shown, although in some cases a curve for a slightly underdamped ($\zeta = .9$) or a slightly overdamped ($\zeta = 1.2$) second-order system gives a better fit. The second-order system parameters of the magnitude plots shown in Figure 4.11 are listed in Table 4.1.

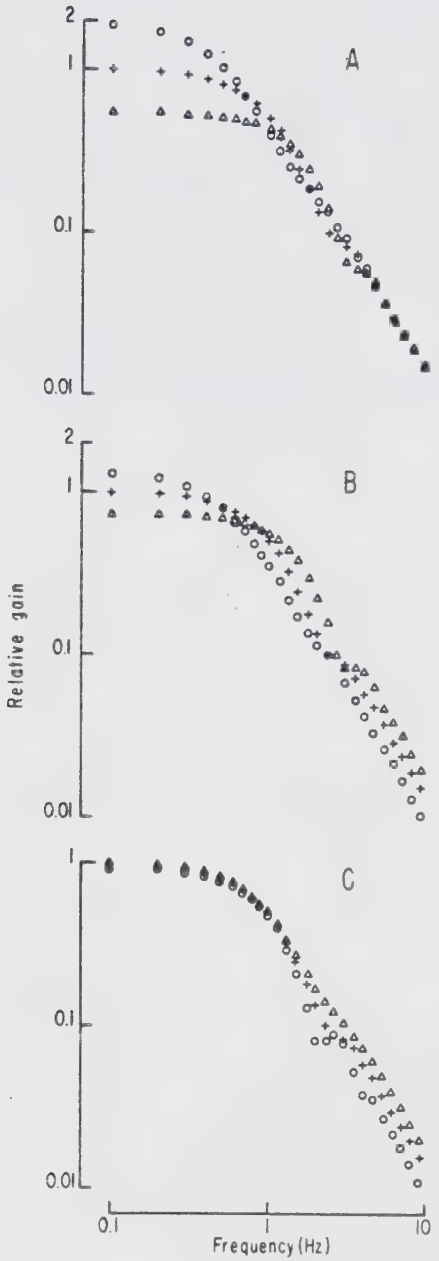

Figure 4.11 The magnitudes of the Fourier transforms of computed isometric twitches after changing different parameters: A) β, B) *SR* and C) *k*. +, 'standard'; Δ, doubling of parameters; O, halving of parameter.

TABLE 4.1 Comparison of the second-order parameters used to describe the frequency response obtained by Fourier transformation of the computed isometric twitches after changing different parameters by a factor of two.

Parameter	Relative G_0 (at .1 Hz)	f_n (Hz)	Estimated ζ
Standard (β, SR or k)	1.0	1.0	1.0
$\beta \times \frac{1}{2}$	1.88	0.56	1.2
$\beta \times 2$	0.54	1.70	0.9
$SR \times \frac{1}{2}$	1.30	0.60	1.2
$SR \times 2$	0.73	1.50	0.9
$k \times \frac{1}{2}$	0.94	0.95	1.0
$k \times 2$	1.02	1.02	1.0

In the twitch tension records for different β (Figure 4.1) and from Figure 4.3, it can also be observed that the characteristics of the twitch are most sensitive to changes in β or *SR*, which change the characteristics in nearly the same fashion. Decreasing β or *SR* produces increase in the 'sluggishness' (increases in T_c and $T_{\frac{1}{2}r}$ in general) of the muscle.

The Fourier transforms are also more sensitive to β and *SR* than to k and are similar in that both β and f_n change in the same fashion. β has a greater effect because its 'standard' value is small (6/sec), and changing it by a factor of two has a relatively greater effect on the isometric twitch, and hence the Fourier transform. In the case of *SR*, C_0 is also changed by the same amount, and this tends to reduce the maximum value of γ and hence the effect of β on the time course of γ. The increased 'sluggishness' as a result of decreasing β or *SR* produces concomitant increase in ζ in general and decrease in f_n. G_0, which measures the area under a twitch record, depends not only on T_c and $T_{\frac{1}{2}r}$, but also on P_t.

As shown in Figure 4.11C, changing k, the stiffness of the series elastic element, by a factor of two has only a minor effect on the Fourier transform. An increase in k has negligible effect on ζ, although G_0 and f_n are slightly increased. Recent studies on the frequency response of isometric soleus muscle in the cat (Mannard and Stein, 1972), however, showed that increasing the stiffness by stretching the muscle increased G_0 and ζ, while f_n was decreased. As shown in Figure 4.5, increasing k results in a greater dP/dt and smaller T_c; these changes are consistent with an increased f_n

observed for the gain curve. Changes in the form of the twitch and the area under the tension curve are probably too small to have a significant effect on the Fourier transform.

CHAPTER 5

METHODS FOR EXPERIMENTATION.

5.1 *Preparation.*

All experiments were performed *in situ* on the sartorius muscle of the bullfrog, *Rana catesbiana*. Preliminary experiments showed that if circulation was kept intact, rapid fatigue could be prevented.

The animal was anesthetized by immersion in a .2% solution of Tricaine Methanesulfonate (Fraser, Vancouver) for 30 to 40 minutes. Narcosis lasted several hours. The distal tendon of the right sartorius muscle was exposed and freed of the adjacent connective tissues. An inextensible steel hook was tied directly to the distal tendon. Particular attention was given to tying the silk thread as tightly as possible very near the ends of the muscle fibers, thereby reducing the series compliance.

The right sciatic nerve distal to the pelvis was exposed from the dorsal surface. The large collateral branch which ultimately innervated the sartorius muscle was cut together with a few millimeters of sciatic nerve. All other muscles of the right limb were denervated. Care was taken to avoid puncturing any major blood vessels. The distal tendon was cut with the steel hook attached to it.

The animal was put into a Perspex trough in its prone position. The trunk was secured by pins forced against its pelvic bone and the legs were clamped to one side of the trough. In this

position, the right thigh was at an angle of approximately 150° from the side of the body and the knee was about 1 cm above the floor of the trough. The thigh muscles near the dorsal surface were spread apart to make a paraffin oil bath in which the nerve was stimulated. Ringer's solution (NaCl, 117 mM; $CaCl_2$, 1.0 mM; K_2HPO_4, 2.4 mM; KH_2PO_4, 0.6 mM; 200 mg% glucose) containing .03% of the anesthetic Tricaine Methanesulfonate was poured into the trough so that the animal was about half immersed. Under this condition the animal could be kept unconscious indefinitely. Limb movements were absent but blood circulation in small cutaneous vessels could be observed. A mixture of 95% O_2 and 5% CO_2 was continuously bubbled into the Ringer's solution. Temperature changes between 3° and 31°C were achieved by circulating water in glass tubings submerged in the bath. The temperature of the circulating water was regulated by a thermistor connected to a cooler (Precision Scientific Lo-Temptrol 154). Temperature of the Ringer was measured to the nearest half degree.

A muscle was stretched from a slack length until it gave a maximum twitch tension. This length (L_0) was not changed throughout an experiment. The weight and L_0 of the eight muscles used in the experiments were 0.97 ± 0.07 gm (mean ± S.D.) and 6.55 ± 0.44 cm, respectively.

5.2 *Stimulation.*

Two types of electrical stimulation of the muscle nerve were used in the experiments. Regular pulse trains were generated by two sets of digital counters and stimulators (Bell and Stein, 1971).

Stimulation using one frequency could be switched to another almost instantly. A random pulse train was produced by adding random noise to a neural analog (Stein and French, 1970) which produced regular pulses in the absence of the random input. These pulses were used to trigger a stimulator. The random pulse train used had a mean rate of 5/sec and the intervals between pulses range from about 25 msec to 1 sec. Stimulus pulses of twice maximal strength and 100 μsec duration were used. The stimulating electrodes were made of a pair of pure silver wires 0.5 mm in diameter.

5.3 *Recording and Analysis.*

Isometric tension was recorded using a Grass FT-03C transducer and a Tektronix 3C66 carrier amplifier. The transducer had a compliance of 1 μ/gm and a resonant frequency of about 330 Hz. Tension records were simultaneously examined on a Tektronix RM565 Dual Beam oscilloscope, monitored on a Hewlett-Packard 7700 pen recorder and stored on magnetic tape (Thermionic Products, T-3000 FM recorder) for analysis.

A Nihon Kohden PC-2A camera was used to photograph isometric twitches in sequence from the oscilloscope face. Peak tension, contraction time and half-relaxation time were measured by hand. Frequency domain analysis was carried out using a LAB-8 computer (Digital Equipment Corporation). Spectral analysis packages (French and Holden, 1971a, b, c) were available to evaluate the frequency response given any analog or digital input and output. In the analysis here the input and output are respectively the random pulse

train and the tension generated.

5.4 *Descriptions of the Frequency Response.*

The parameters used to specify the gain curve of the frequency response of a second-order system are given in Chapter 4. These are zero frequency gain (G_0), natural frequency (f_n) and damping ratio (ζ).

The data points for the estimated frequency response were converted to units (gm-sec)/impulse at a certain frequency, displayed on the screen of a storage unit (Tektronix 601) and photographed using a Polaroid camera.

A family of 16 frequency response curves for the gain of a linear, second-order system was constructed on a transparency with logarithmic axes having the same scales as those of the Polaroid photographs. By sliding the transparency over the photographs, the curve providing the best fit to the gain data was selected and the parameters G_0, f_n and ζ could be specified to an accuracy of about 15%.

A coherence function which gives a normalized measure of the extent to which the linear frequency response function accounts for the total response of the system at any given frequency was also printed out. A coherence of 1 would indicate the behaviour of the muscle could be described by a linear, time-invariant system with no noise or other extraneous inputs. The data were also printed out using a teletype machine.

CHAPTER 6

RESULTS OF EXPERIMENTATION.

6.1 *Effects of Tetanus and Temperature on the Characteristics of the Isometric Twitch.*

(i) Peak Twitch Tension.

The effect of tetanus on the isometric twitch tension obtained by stimulating the muscle nerve was studied in the temperature range $3\frac{1}{2}$ to 30°C. A tetanus of a few seconds duration produced a potentiation of the isometric twitch (a greater P_t and a longer time course), but the amount of potentiation was not maximal until some time after the end of the tetanus. Figure 6.1 shows the isometric twitch tension record of one typical experiment at 20°C. Immediately after the tetanus, peak tension rose nearly linearly to a peak and decayed with an approximately exponential time course. This potentiation after a delay was also observed in frog sartorius by Connolly, Gough and Winegrad (1971), when the muscle was stimulated directly. In the record shown in Figure 6.1, the rising phase of potentiation lasted about 16 seconds, and was followed by a falling phase that lasted for over 3 minutes.

The effect of tetanus produced by about 250 stimuli was observed at any one temperature. At temperatures above 10°C, a stimulation rate of 50/sec was used, while at lower temperatures, 33/sec was used. These stimulation rates were adequate and satisfactory to produce potentiation in these temperature ranges. Close and Hoh (1968b) showed that the total number of stimuli is the more important

Figure 6.1 The effect of tetanus on the isometric twitch tension of a sartorius muscle at 20°C. Rate of stimulation was 30/min following a 5 sec tetanus at 50/sec.

parameter in determining the degree of potentiation in rat fast skeletal muscle. Preliminary studies indicated that this was also true in frog muscle fibers, although at lower temperatures (below 10°C), a lower rate of stimulation (33/sec) seemed to be more effective.

To produce twitches, the nerve was stimulated at a rate of 30/min. At lower temperatures, even this rate would somewhat alter the characteristics of the twitch; therefore, only about 10 twitches were elicited before the tetanus. The isometric twitch immediately before the tetanus was referred to as the 'control' twitch. After a temperature change, about 30 min were allowed for the muscle to equilibrate with the new temperature.

The separation of the post-tetanic potentiation into two phases was a common feature for a wide range of temperatures, although at temperatures greater than 25°C the rising phase was usually absent. This means that maximal potentiation was observed in the first few seconds after a tetanus. In the case where a rising phase was present, the value for peak tension at 2 sec after a tetanus was not always the same as the pretetanic value; sometimes it was higher and occasionally lower.

Figure 6.2A shows, in a linear plot, peak tension of the isometric twitches in the rising phase at time t after the tetanus at different temperatures for one muscle. The points at any one temperature are fitted by straight lines. P_t of the 'control' twitches are also shown. The peak tensions of the maximally potentiated twitches are shown separately in Figure 6.4.

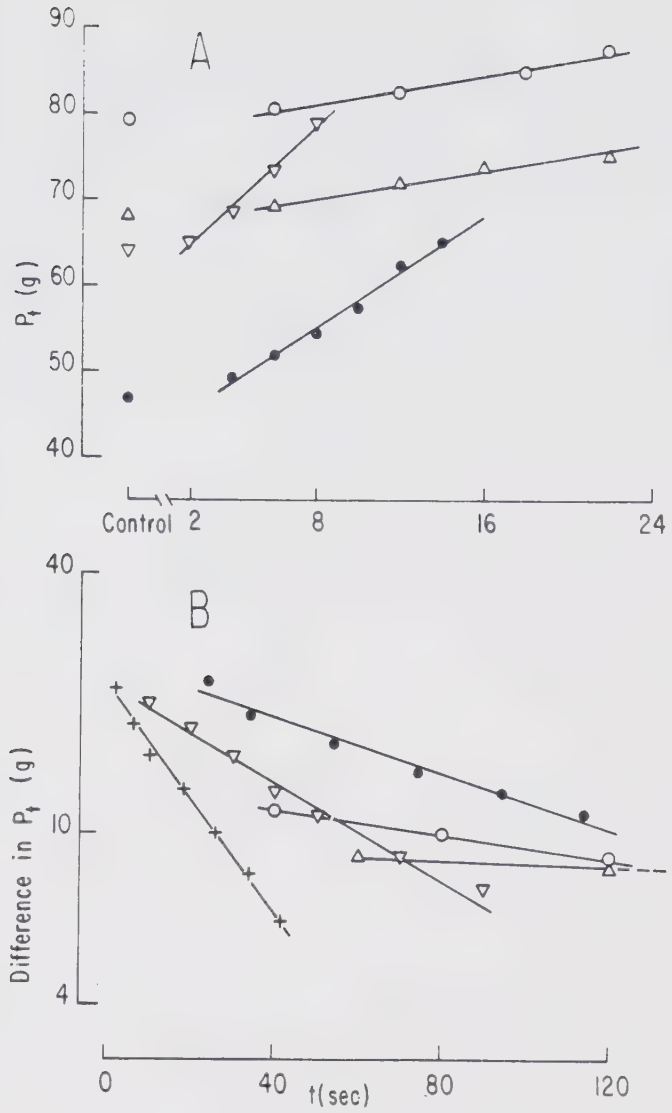

Figure 6.2 A. Linear plots of time course of rising phase of potentiation at different temperatures. Peak tension, P_t, is shown at time t after a tetanus. 'Control' indicates pretetanic values. The last point of each set has a value about 80% of the maximum value attained.
B. Semi-logarithmic plots of time course of falling phase of potentiation. Difference in peak tension is shown at time t after a tetanus. The first and last points of each set have respective values of about 80% and 30% of the maximum difference in P_t. Temperatures: 4° (Δ), 9½° (○), 17° (●), 24° (▽) and 29½°C (+). Only two points are shown for the falling phase at 4°C because of the minimum difference (1.25 g) distinguishable in the measurement; two other points were also used to obtain the fitted line.

Figure 6.2B shows, in a semi-log plot, the difference in P_t of the twitches during the falling phase. The difference in P_t was usually obtained by subtracting P_t of the 'control' twitch from that of the twitches in the falling phase. At higher temperatures, it was not unusual for the post-tetanic P_t to fall to a lower value after the potentiation and increase again, or remain at that level. In this case, the difference was obtained by subtracting the P_t in the 'depressed' twitch. The differences of peak tension obtained in this way for the twitches in the falling phase are shown in Figure 6.2B. The time scale indicates the time course of the fall t sec after the tetanus.

Different rate constants (in units of 'gm/sec' for the rising phase and 'sec^{-1}' for the falling phase) were obtained from the fitted lines on the two phases. The rate constants of change of the rising phase of potentiation at different temperatures for four muscles are shown in Figure 6.3A, in a semi-log scale. Temperature changes for the four preparations were as follows: increasing temperature in two muscles, decreasing in one, and increase followed by a decrease in the fourth. Data were obtained only for temperatures below 25°C because at higher temperatures, maximal potentiation was usually reached in a few seconds after a tetanus and too few twitches were obtained to give an accurate measure of the rising phase. Below 25°C, the rising phase of potentiation is fitted by a straight line (corr. coeff. = .97) and has a Q_{10} of about 2.7 (S.D. = .17).

Figure 6.3B shows the rate constants of differences in P_t of twitches in the falling phase in the same experiments. The points

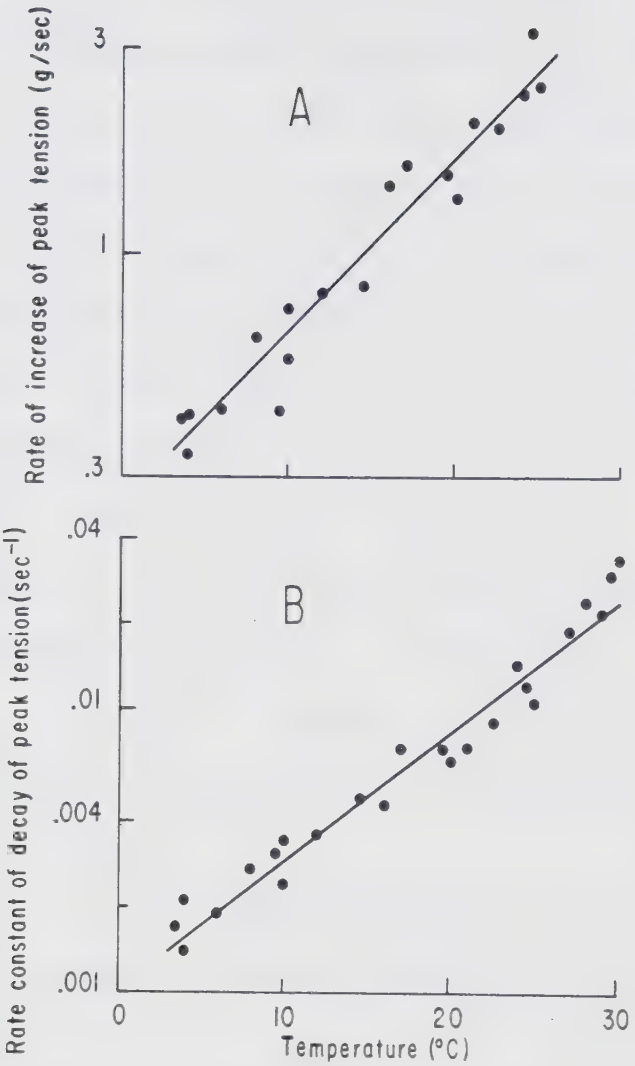

Figure 6.3 A. Semi-logarithmic plot of rate of increase of peak tension (rising phase of potentiation) at different temperatures.
B. Semi-logarithmic plot of rate constant of decay of difference of peak tension (falling phase of potentiation) at different temperatures. Data are obtained from the fitted lines shown in Figure 6.2 and from three other experiments.

are also fitted well by a straight line (corr. coeff. = .98); a Q_{10} of 2.9 (S.D. = .18) is obtained from the fitted line.

Figure 6.4 compares the peak tension of the 'control' and the maximallypotentiated twitch at different temperatures, obtained from the same experiments. The two sets of points are fitted by straight lines. Since the weight and optimum length of any of the muscles used deviated within 10% of their mean values (see Chapter 5, Section 5.1), and the error of measurements in the experiments was estimated to be about ±15%, the factors of weight and length were not considered.

Several results are obvious from Figure 6.4.

(1) Tension varies over a wide range of temperature.

(2) Peak tension decreases with temperature.

(3) For the 'control' (pretetanic) twitch, peak tension decreases by about 49% for a temperature change from $3\frac{1}{2}$ to 30°C.

(4) For the same temperature change, peak tension of the maximally potentiated twitch decreases by about 22%.

The decrease of P_t with temperature was observed for frog muscles in the range 0° to 20°C (Hill, 1951c; Kelley and Fry, 1958) whereas Jewell and Wilkie (1958) reported both an increase and decrease in P_t.

(ii) Contraction Time, T_c, and Half-Relaxation Time, $T_{\frac{1}{2}r}$.

The contraction time and half-relaxation time of the 'control' twitch were greatly decreased with an increase in temperature.

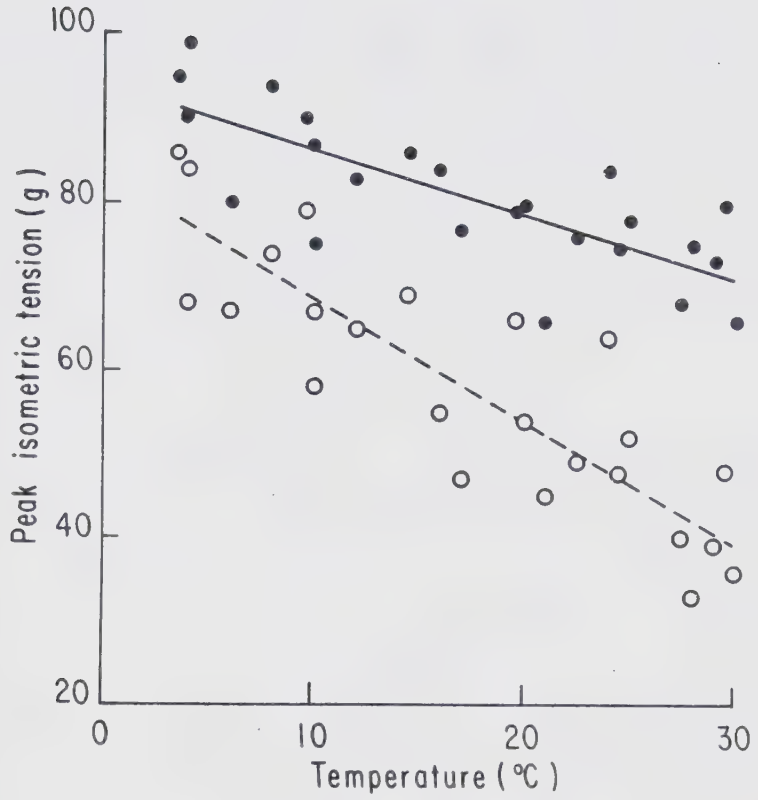

Figure 6.4 The relation between temperature and peak tension in the 'control' twitch (O) and the maximally potentiated twitch (●). Each set of points is fitted by straight lines (least square method). Tension decreases at a rate of 14.5 g/10°C from 83.0 g at 0°C in the 'control' twitch (---) and 7.6 g/10°C from 93.7 g at 0°C in the maximally potentiated twitch (——). Data from four sartorius muscles.

Tetanus also produced an immediate increase in both T_c and $T_{\frac{1}{2}r}$. The values then returned very slowly with a variable time course to their control values.

Since T_c and $T_{\frac{1}{2}r}$ represent the duration of tension change of the contraction and relaxation phase respectively, their reciprocals indicate rates of change. The reciprocals $1/T_c$ and $1/T_{\frac{1}{2}r}$ were referred to as the contraction rate and relaxation rate respectively and were plotted in Figure 6.5 at different temperatures for the 'control' twitch and the maximally potentiated twitch from the same experiments as in the previous section.

Each set of data points is fitted by straight lines (all have corr. coeff. > .9). If semi-logarithmic fit were used instead, the correlation coefficients are not significantly different and the Q_{10}'s so obtained are in the range 1.9 to 2.2. Using the slopes of the fitted lines, the ratio $T_{\frac{1}{2}r}/T_c$ for the whole temperature range is found to be equal to 1.3 for the 'control' twitch whereas a value of 2.0 is obtained for the maximally potentiated twitch.

Using the values from the fitted lines, it can be observed that the ratio $T_{\frac{1}{2}r}/T_c$ is increased by tetanus from 1.8 to 1.9 at 10°C, and from 1.5 to 2.2 at 25°C. In other words, although both $T_{\frac{1}{2}r}$ and T_c are increased by the tetanus, the percentage increase in $T_{\frac{1}{2}r}/T_c$ is greater at higher temperatures.

6.2 *Effects of Temperature and Tetanus on the Frequency Response of the Isometric Muscle.*

The response of the isometric muscle to nerve stimulation was

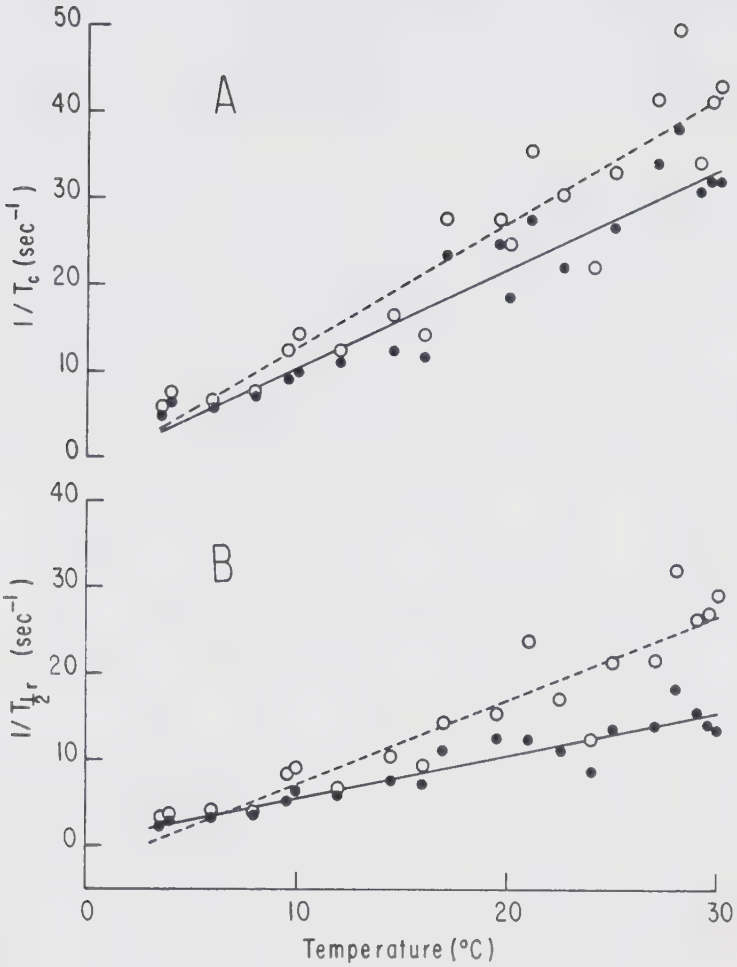

Figure 6.5 The relation between temperature and A) contraction rate, $1/T_c$ and B) relaxation rate, $1/T_{\frac{1}{2}r}$ in the 'control' twitch (--O--) and the maximally potentiated twitch (—●—). Data from four sartorius muscles.

studied in the frequency domain. Figure 6.6A shows an isometric twitch of sartorius obtained by supramaximal stimulation of the muscle nerve at 20°C. The magnitude of the Fourier transform computed from this twitch by the method of French and Holden (1971a, b, c) is shown in Figure 6.6B.

The fitted curve in Figure 6.6B is the response which would be expected for a critically-damped, second-order system which has a transfer function given previously by equation (4.3) in Chapter 4. The critically-damped, second-order representation is extremely good at this temperature, although in some cases a curve for a slightly overdamped second-order system gives a better fit. Underdamping is not observed in the frequency response of real muscles. The frequency response has a shape which resembles the Fourier transform of the 'standard' twitch described in the model previously.

Figure 6.6C compares the observed phase angles with those derived from the frequency response curve in Figure 6.6B. The deviation from the predicted angle results from the delayed response of the muscle to stimulation. Time delay does not affect the magnitude of the frequency response, but increases the phase angle in proportion to its frequencies (Ogata, 1970). However, when the observed 6.5 msec latency between muscle nerve stimulus and muscle response is taken into account, the dashed curve in Figure 6.6C is obtained. This indicates that if the stimulus is applied to a model with the fitted frequency response, the output in the time domain closely resembles the tension record obtained experimentally. The latency in the experiment results mainly from nerve conduction,

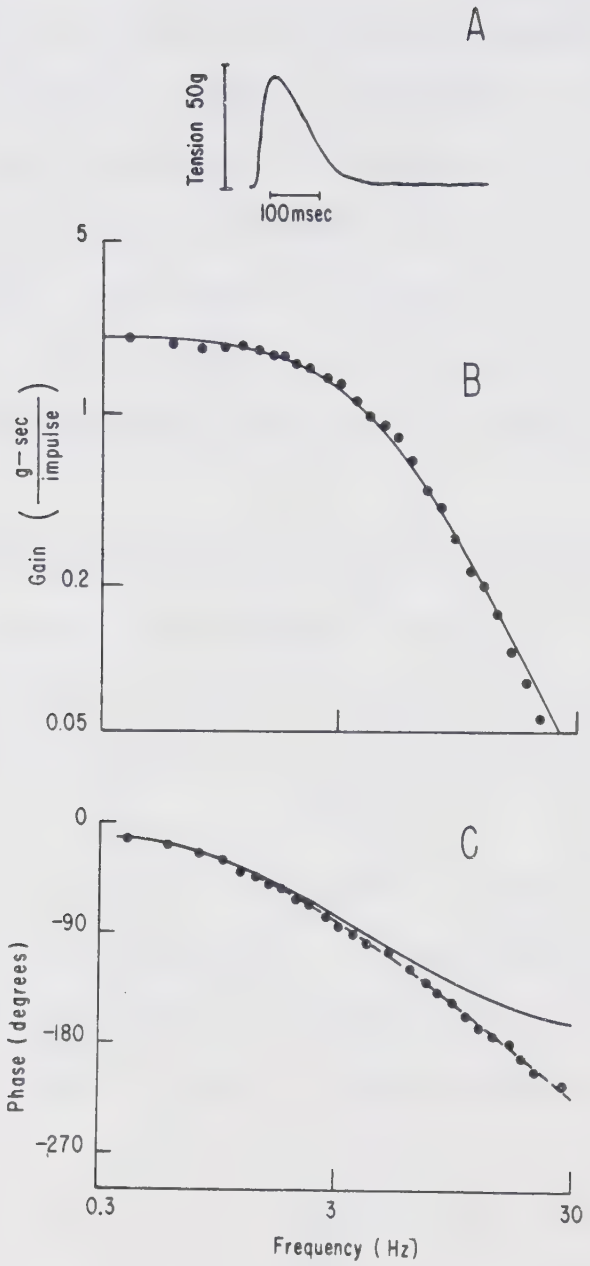

Figure 6.6 Isometric twitch (A) of sartorius muscle obtained by stimulation of the muscle nerve at 20°C. The magnitude (B) and phase (C) of the frequency response of sartorius muscle obtained by Fourier transformation of the isometric twitch. Further explanation in text.

synaptic transmission and excitation-contraction coupling.

As pointed out earlier, the frequency response computed from the isometric twitch is adequate to describe the responsiveness of the muscle to nerve stimulation if the system is linear. In the nervous and muscular systems, many non-linearities exist. Moreover, nerve impulse trains in motor axons are variable in time and asynchronous. This can be approximated to some extent by applying random stimulation to the muscle nerve and recording the partially-fused or unfused tension.

The effect of tetanus on the frequency response was determined using this method in the temperature range 3° to 31°C. The stimulation sequence was as follows. A random pulse train that had a mean rate of 5/sec was applied to the muscle nerve for 30 sec and tension was recorded simultaneously (referred to as the 'control' record). At least 15 min were then allowed to elapse. This permitted the effect of the random stimulation to subside and the muscle to return to its resting condition. A second random train was then applied immediately after a tetanus elicited by about 250 stimuli in a way given in Section 6.1; the tension recorded was referred to as the 'post-tetanic' record. In all the records used for analysis, the peak tension of the isometric twitch before the first random stimulation and that before the tetanus did not differ by more than 20%. Temperature change was achieved in a manner given before.

Figure 6.7 shows the frequency responses obtained from a 'control' and a 'post-tetanic' record at 16°C. Tetanus produces an increase in G_0 by about 50% over the 'control'. The natural frequency,

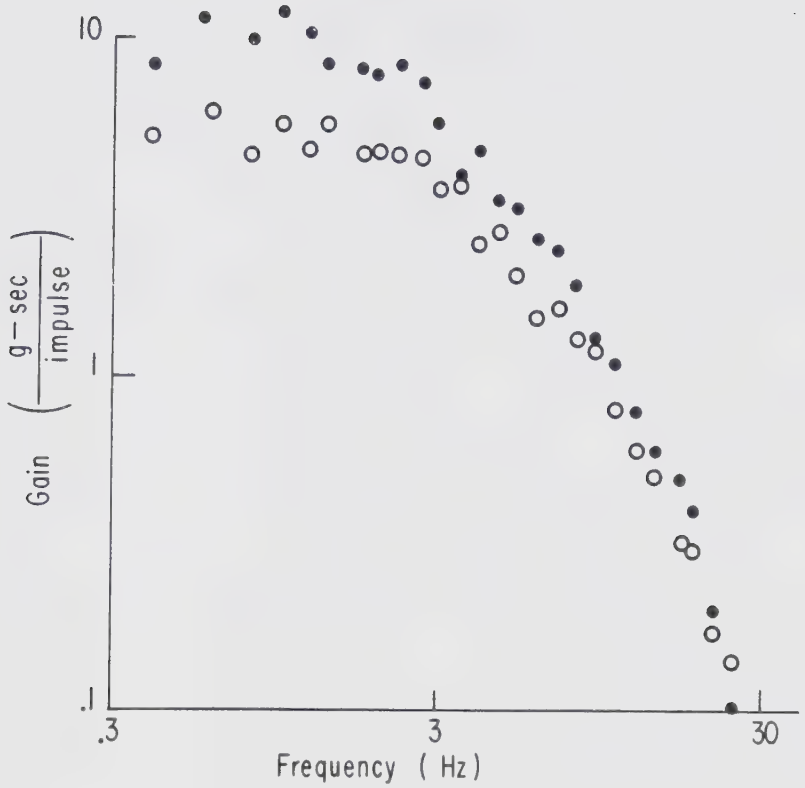

Figure 6.7 The frequency response of the sartorius muscle obtained by random stimulation in a 'control' period (O) and after a 5 sec tetanus at 50/sec (●) at 16°C.

f_n, decreases from 4 to 2.9 Hz. A slight increase is also observed in ζ.

From the studies described in the previous section, it was observed that maximal potentiation defined by P_t usually occurred in the first few seconds after a tetanus for temperatures greater than 25°C and at 30 to 40 sec at lower temperatures (below 10°C). In the present experiment when a random train of 30 sec duration was applied after a tetanus, tension was recorded when post-tetanic potentiation might not be maximal, if the temperature were below 10°C. Even with this duration, the stimulus train produces some potentiation of the isometric twitch following the 'control' random stimulation. The effect on the isometric twitch is however small compared with that produced by a tetanus. A random train of longer duration would bring about an early fatigue, especially at higher temperatures.

Four muscles different from those described in Section 6.1 were used to examine the effects of tetanus and temperature on the frequency response. Figure 6.8 shows the second-order parameters G_0, f_n and ζ of the frequency responses obtained from a typical experiment. Several results follow from Figure 6.8:

(1) In the 'control frequency response, raising the temperature decreases G_0 and ζ, and increases f_n.

(2) G_0 is greater in the 'post-tetanic' response; the percentage-increase becomes smaller as temperature is raised.

(3) f_n is smaller in the 'post-tetanic' response; the percentage-decrease is about 30% at all temperatures.

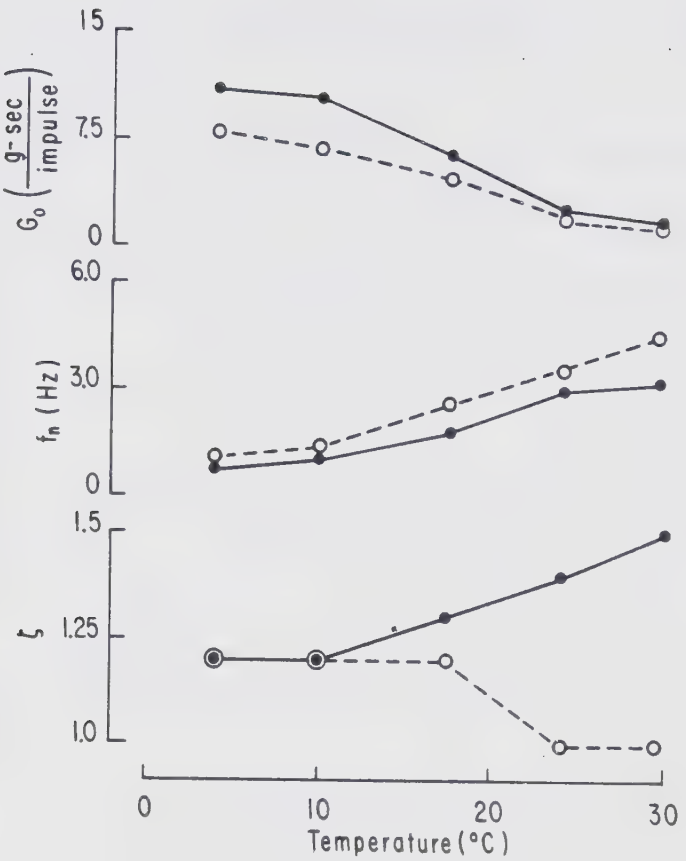

Figure 6.8 The influence of tetanus and temperature on the second-order parameters: G_0, zero-frequency gain; f_n, natural frequency, and ζ, damping ratio (shown in ordinate) used to describe the frequency response of the sartorius muscle obtained by random stimulation of the muscle nerve. O, 'control' and ●, post-tetanic frequency response parameters. Data from one muscle.

(4) A nearly linear relationship exists between f_n and temperature.

(5) Differences in ζ between the 'control' and the 'post-tetanic' response become marked at higher temperatures.

These results are representative of the experiments performed. This is also true whether temperature is raised or lowered. At higher temperatures, G_0 was sometimes found to be smaller in the 'post-tetanic' response. This was probably due to muscle fatigue. At lower temperatures, G_0 was always greater in the 'post-tetanic' response; ζ did not change appreciably although both T_c and $T_{\frac{1}{2}r}$ were increased after a tetanus. The coherence function is greater than .8 for most of the frequencies(in the frequency response); this indicates the nerve-muscle system is nearly linear with this mean rate (5/sec) of stimulation.

The most consistent changes were observed in f_n. The rates of change of tension defined by $1/T_c$ and $1/T_{\frac{1}{2}r}$ vary almost linearly with temperature (see Figure 6.5). Similar relationship is also found between f_n and temperature, as is obvious from Figure 6.9. The data were obtained from four muscles and were fitted by a straight line (corr. coeff. = .98). Similar results were obtained for f_n in the 'post-tetanic' response, although it was decreased at any temperature and the rate of change with temperature was also smaller.

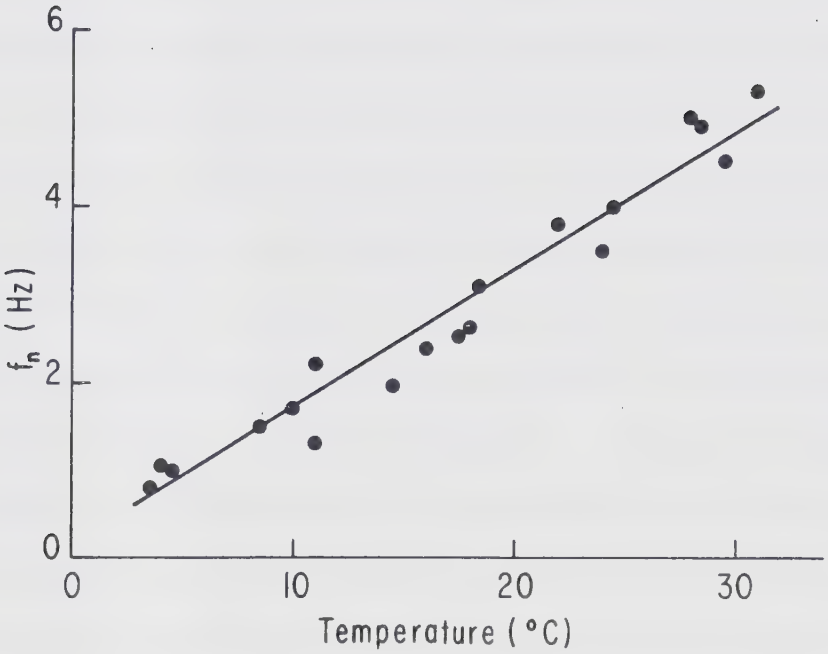

Figure 6.9 The relation between temperature and natural frequency, f_n, of the sartorius muscle. The fitted line intercepts the ordinate at .12 Hz (0°C); f_n increases at a rate of 1.5 Hz/10°C. Data from four muscles.

CHAPTER 7

DISCUSSION

The characteristics of an isometric twitch are manifestations of the underlying chemical and mechanical processes. Hill (1951c) suggested that the maximum tension was reached as a balance between two opposing processes, internal shortening (mechanical) on the one hand, and decay of the active state (chemical) on the other. In the model presented here, the sliding filament theory forms the basis of the mechanical process while an activation mechanism depending on Ca^{++} constitutes the chemical process. The parameters governing the two processes in the model are many and are subjected to change under different circumstances. In this discussion, an attempt is made to elucidate the relative importance of the parameters from their influence on the characteristics of the isometric twitch, and to relate them to the structural and functional differences observed in skeletal muscles. The effects of tetanus and temperature on the characteristics of an isometric twitch observed in the studies here and elsewhere are also discussed in light of the model and other experimental evidence.

7.1 *Parameters of the Sliding Filament Model.*

The parameters in the model have different, and characteristic, effects on the form of an isometric twitch. A comparison of Figures 4.3 and 4.4 show that the constraints f_1, g_1 and g_2 for the making and breaking of cross-bridges have a smaller effect on the time course of the twitch than the parameters in the activation factor. f_1 has a

similar but greater effect on the contraction phase than g_1, while they have opposite effects on the relaxation phase. This seems surprising because f_1 represents the making, while g_1 the breaking of cross-bridges. One possible explanation is as follows. In considering the time course of tension development, we must take into account both the rate of making of cross-bridges and the number of cross-bridges available. A faster rate of breaking of cross-bridges (a larger g_1) will mean that a greater number of cross-bridges are available for making. The greater rate of cycling of cross-bridges permits the series elasticity to be extended more rapidly and hence lead to a greater rate of tension development. The relaxation phase is, however, a true indication of the opposite nature of f_1 and g_1. On the other hand, g_2 has negligible effect on the form of a twitch. This is because the value of g_2 is large compared with either f_1 or g_1, so that a change by a factor of four has little overall effect on the time course of a twitch. The number of cross-bridges made in the region where g_2 has a non-zero value is small, and the probability of the cross-bridges to be made in this particular region is also low under the isometric condition (Huxley, 1957). Although internal shortening occurs in the present model, and it is thus not a truly isometric condition, the velocity of shortening is still too small to have a great effect on the interaction of cross-bridges. The net effect is that f_1, the rate of making of cross-bridges has a greater influence on the characteristics of the isometric twitch than g_1.

7.2 *Parameters of the Activation Factor.*

The activation mechanism is developed for frog sartorius muscle at a temperature of 0°C. Unless stated otherwise, any change would be discussed in reference to the 'standard' activation and 'standard' muscle at this temperature.

Among the parameters in the activation factor, β has the greatest effect of all parameters on the relaxation phase of the isometric twitch. β is assumed to be the rate of release of Ca^{++} from the bound state in the myofilaments. It is generally believed that relaxation results from the removal of Ca^{++} from the myofilament space by a process which resembles Ca^{++}-chelating by EDTA (Bozler, 1954; Watanabe and Sleater, 1957; Ebashi, 1960, 1961b), or by a 'relaxing factor' associated with the sarcoplasmic reticulum if the latter structure exists (Hasselbach, 1964). Therefore, β can also be assumed to be the rate of removal or re-uptake of Ca^{++} by the sarcoplasmic reticulum. Whether a clear distinction can be made between the two processes is not known.

Experimental evidence suggests that potentiation of the isometric twitch results from the prolongation of the active state (Goffart and Ritchie, 1952; Ritchie and Wilkie, 1955). In the model, decreasing β increases the magnitude and prolongs the time course of the activation factor (see equation (2.12)). The time course of the activation factor is similar to the active state curves studied by many workers (Hill and Macpherson, 1954; Macpherson and Wilkie, 1954; Close, 1965). This permits a greater number of cross-bridges to interact and a longer time for internal shortening so that not only a

greater peak tension is attained, but the time course of relaxation is also significantly prolonged.

Similarly, α , the rate of calcium binding by the myofilaments, is not necessarily distinguishable from the rate of calcium release by a stimulus. In the model, changing α has a relatively small effect on the characteristics of the isometric twitch. The time course of the activation factor in the model is determined by both α and β. Since the 'standard' value of α (65/sec) is already large compared with that of β (6/sec), changing it (up to a factor of four) does not alter the time course of the activation factor appreciably, and hence the isometric twitch.

7.3 *Activation Mechanisms in Fast and Slow Muscle Fibers.*

It has been usual to classify muscle fibers according to their speed of contraction, and relaxation; their motor innervation; and in some cases to their histochemical properties. Histochemical studies have revealed there are in general three types of extrafusal fibers (fast, intermediate and slow) in mammalian striated muscles (Nachmias and Padykula, 1958; Stein and Padykula, 1962; Romanul, 1964; Henneman and Olson, 1965). Studies at the motor unit level have shown that a smaller contraction time is associated with a larger diameter of the motor axon and muscle fiber (Wuerker, McPhedran and Henneman, 1965; Appleberg and Emonet-Denand, 1967; Burke, 1967). Mammalian extrafusal muscles are generally composed exclusively of twitch fibers, which conduct action potentials.

Amphibian skeletal muscle is generally regarded as being

composed of two types of muscle fibers: 'fast' and 'slow'; these are in turn innervated by the large- and small-nerve motor system (Tasaki and Mizutani, 1944; Kuffler and Gerard, 1947). A fast fiber can conduct action potentials and produce a twitch, whereas slow fibers show no regenerative electrical activity and respond to local depolarization with a local contraction (reviews: Peachey, 1961, 1968). Slow fibers also have a lower fusion frequency and produce a tetanic tension which is better maintained than the fast fibers. Lannergren and Smith (1966) described five fiber types in the iliofibularis muscle of *Xenopus laevis* although the different types were classified into three main groups: two 'fast' and one 'slow' with overlapping functional properties. Studies on the motor units in the same muscle have shown both the 'fast' and 'slow' types can be subdivided (Smith and Lannergren, 1968). These authors also point out there seems to be a relationship between the properties of the motor unit and the diameter of its motor axon.

Another difference between fast and slow muscle fibers resides in the sarcotubular system. It is generally thought that the slow fiber lacks a proper T-system (for review: Hess, 1967, 1970). Evidence from Flitney (1971) shows that frog slow fibers have an elaborate T-system network and sarcoplasmic reticulum. Their relative volumes are, however, about half as much as in a twitch fiber. Costantin, Podolsky and Tice (1967), using 'skinned' preparations, showed that the sarcoplasmic reticulum in a slow fiber is capable of accumulating calcium, although it appears to be less effective in doing so than that of a twitch fiber.

Based on this evidence, two assumptions are made for the

activation mechanism in a slow muscle. The amount of calcium released per fiber volume by a stimulus is smaller, and so is the rate of calcium uptake by the sarcotubular system. In the model, this would be a reduced C_0 and β. As shown in Chapter 4, this gives a twitch which has a smaller P_t, a lower dP/dt, and a longer T_c and $T_{\frac{1}{2}r}$. A tetanus which is produced by reducing C_0 and β also has a smaller maximum tension which is better maintained, a longer time to peak and a lower fusion frequency. These types of response are observed experimentally in some slow fibers (Smith and Lannergren, 1968; review: Hess, 1970).

Another fundamental difference between 'fast' and 'slow' fibers is also found for the maximum rates at which the sliding filaments can interact. Measurement of the heat production during a tetanus (Floyd and Smith, 1971) and of the shortening velocity of the sarcomeres in 'skinned' fibers (Costantin, Podolsky and Tice, 1967) and intact fibers (Peachey, quoted by Costantin *et al.*, 1967) show that these processes are probably 10 to 30 times slower in the slow fiber. The myofibrillar ATP-ase activity, the property which ultimately determines contractile performance (Barany, 1966), has also been shown, by histochamical means (Engel and Irwin, 1967), to be low in the slow fiber. It is, therefore, reasonable to suppose that since its myosin is capable only of a comparatively slow rate of activity, the rate at which it must be supplied with calcium need not be so great as in a twitch fiber. The rate at which the sliding filament can interact is probably equivalent to the constraint f_1, the rate of making cross-bridges in the sliding filament model. Decreasing f_1 does give a twitch similar to that found in a muscle which has a

poorly-developed sarcoplasmic reticulum and again shows that f_1 is one of the more important parameters in the model.

There is a general, though not invariable correlation between speed of contraction and relaxation of striated muscle fibers, and the volume and organization of the T-system and sarcoplasmic reticulum (review: Smith, 1966). In the attempt to model slow muscle, excitation-contraction coupling does not enter explicitly into the model. Much of the behavior observed experimentally in slow muscles is, however, obtained just by reducing C_0 and β. In a real muscle, it is possible that the slowness of the activity cycle of the slow fibers reflects not a radically different excitation mechanism (to the extent of Ca^{++} binding and removal), but a relatively more important Ca^{++} exchange between the surface membrane and the exterior of the fiber (Makinose and Hammad, cited by Hasselbach, 1964). Even in exceptional cases such as the asynchronous flight muscles of certain insects (Pringle, 1965) which have a well-developed T-system but a reduced sarcoplasmic reticulum, some of their behavior could be obtained by modifying an activation mechanism which is very similar to that in the present model (Julian, 1969).

In the extreme case where a 'threshold' is included in the activation factor, the effects on a twitch or tetanus obtained by reducing C_0 and β are exaggerated. If only one stimulus is given, no contraction is produced. These responses are observed in a true slow fiber. The tetanus obtained by stimulating '$\frac{1}{2}SR$ (reducing C_0 and β by half) + threshold' muscle (Figure 4.8) at a rate of 20/sec has a tension which is better maintained than that in the 'standard'

muscle although it still declines at a relatively fast rate. Kuffler and Vaughan-Williams (1953) observed that with a stimulation rate of 4/sec at 20°C the tension record of frog iliofibularis (a muscle considered to be 'slow') is fused and maintained for many seconds. The decline of tetanic tension in the model results from an absence of replacement of the Ca^{++} store, whereas in a real muscle the Ca^{++} store is continuously replenished. Winegrad (1970) reported that only 2 sec after a short tetanus, some of the calcium released was already transported back to the terminal cisternae, the main release sites. This feature has not been included in the model, because the process involving the redistribution of Ca^{++} is complex as will be obvious later.

Other characteristics of a muscle are demonstrated from its frequency-tension curve (Matthews, Rack and Westbury, 1969). This curve depends to a certain degree on the way in which tension is calculated. The curves for the 'standard' and the '$\frac{1}{2}SR$' muscle rise very sharply without a sigmoid shape (Figure 4.9). This is partly because of the activation itself which is modelled at 0°C. At this temperature, the twitch/tetanus ratio is almost one and the average twitch tension is relatively high. Fusion frequency is low and the tetanus is almost completely fused at only 10/sec for the 'standard' muscle (Figure 4.7). The '$\frac{1}{2}SR$ + threshold' frequency-tension has a sigmoid shape and represents the usual response found in real muscles (Matthews, 1959; Kernell, 1966).

7.4 *Series Elastic Element.*

The series elastic element constitutes an integral part of the mechanical process of contraction. Hill (1951a) noted that an added compliance reduced the peak tension and the maximum rate of tension development while contraction time was increased. The changes observed in the twitch by changing k are similar to those observed by Hill (1951a) in his experiments, although the contraction time in the model is much more sensitive to changes in k (Figure 4.6). In face of the closeness of fit in P_t and dP/dt, between experimental and theoretical data, the reasons for the discrepancy in T_c are unknown at present.

Increasing k (reducing the compliance), however, affects the force generation process in a different way. As shown in the Fourier transform of the isometric twitch (Figure 4.11C), an increase in k has negligible effect on ζ, although G_0 and f_n are slightly increased. Recent studies on the frequency response in the cat (Mannard and Stein, 1972), however, showed that increasing the stiffness by stretching the muscle increased G_0 and ζ, while f_n is decreased. In a real muscle, there is ample evidence that stretching affects the activation mechanism. The prolongation of the active state by stretching may result from an increase in the amount of activator released, a reduction in the rate of removal of activator, or a combination of both (Close, 1972; review: Sandow, 1965). In some instances, stretching a muscle can release sufficient calcium to produce a contraction (Armstrong, Huxley and Julian, 1966; Pringle, 1968; Brown, Goodwin and Matthews, 1971). The results from the model are as expected for a simple second-order system

and are also obvious from the tension curves for different k (Figure 4.5). For example, an increased k would shorten the time course of contraction and relaxation; this gives a larger f_n in the frequency response. In the model, since the activation mechanism is independent of any change in k, the discrepancy of the results from those of Mannard and Stein (1972) seems to lie in some kind of feedback which is absent in the model, but present in a real muscle. A strict comparison is probably not justified because the frequency response obtained by these authors was by random stimulation of the cat soleus muscle at 37°C whereas that in the model is just the Fourier transform of a twitch at 0°C, although the second-order representation is good for either one.

Temperature is another factor which may affect the compliance in elastic elements of the muscle. Walker (1951) found that extensibility and stress relaxation are only slightly affected by as much as 20°C change of temperature in unstimulated rat triceps surae. These findings indicate that the elastic properties of resting rat muscle are changed very little by change of temperature. Hill (1951a) showed that the compliance of toad sartorius, as indicated by resistance to stretch, is only slightly increased by as much as 20°C decrease of temperature. Jewell and Wilkie (1958), in a very carefully controlled series of experiments, found that very large decreases of temperature produced only small increases of extensibility, as observed in the load-extension curve for series elastic elements in toad sartorius. It must be recognized that compliance does play a role in limiting the rate of tension development. However, since compliance of series elastic elements seems to be rather temperature-independent, it is

unlikely that more than a small part of the marked reduction of the rate of tension development induced by cooling can be attributed to an increase of compliance of the series elastic elements. Also, for changes in other characteristics of isometric twitches which follow temperature change, an explanation must be sought elsewhere, for example, in the force generation and the activation processes.

7.5 *Effects of Tetanus and Temperature on the Characteristics of the Isometric Twitch.*

(i) Peak Twitch Tension, P_t.

Tetanus and temperature produce systematic changes in the post-tetanus twitch tension P_t. The effect of temperature on the magnitudes of twitch and tetanic tension development of skeletal muscle vary widely as reported from different laboratories. Jewell and Wilkie (1958) observed both an increase and a decrease of twitch tension in frog sartorius with an increase of temperature. Winchester (1969) reported an increase in twitch tension from 10° to 20°C and a decrease above 20°C in the normal rat diaphragm muscle, while an increase was observed from 10° to 35°C in the denervated muscle. In the lower range of temperatures (below°20 C), most workers report a consistent decrease in twitch tension, and increase in tetanic tension with an increase of temperature (Hill, 1951c; Kelley and Fry, 1958; Walker, 1949; Close and Hoh, 1968a). In the experimental studies reported in this thesis, it was observed that the peak tension in the control twitch decreases by about 49% with a change of temperature from $3\frac{1}{2}$ to 30°C. However, P_t in the maximally potentiated twitch decreased

only by 22%. This means that the tension in the potentiated twitch is almost doubled at higher temperatures, while at $3\frac{1}{2}°$ the increase over the 'control' is only about 15%.

Close and Hoh (1968a) observed that with rat extensor digitorum longus (EDL, considered to be a 'fast' muscle), P_t in the 'control' isometric twitch decreased by about 40%, while that in the maximally potentiated twitch remained almost at the same value with a temperature change from 20° to 35°C. P_t in the soleus (SOL, a 'slow' muscle), however, increased by only a few percent with the same temperature change. There is little or no post-tetanic potentiation in EDL at 20°C and P_t at that temperature is about the same as the peak tension of the maximally potentiated twitch at other temperatures between 20° and 35°C. The increase in tetanic tension, however, is considerable (about 30%) in EDL, but is very small in SOL with the same temperature increase. A similar degree of maximum post-tetanic potentiation has been observed in cat fast muscles at 37°C and frog twitch muscle at about 20°C and has been shown to occur in single muscle fibers (Brown and von Euler, 1938; Ramsey and Street, 1941). A possible explanation suggested by Close and Hoh (1968a) for the differences in temperature dependence of frog twitch muscle and rat EDL muscle on one hand and rat SOL on the other is that a decrease in temperature decreases the intrinsic speed of shortening and the rate of removal of activator in all these muscles, thereby causing an inversely proportional increase in P_t (Close, 1965). The decrease in temperature, however, also increases P_t of frog twitch muscle (for example, the sartorius) and adult rat EDL, in much the same way that repetitive stimulation in-

creases it, by increasing the degree of activation of individual muscle fibers with little or no change in the time course of the response.

The above scheme offers a good explanation for the results obtained here and elsewhere. The time course of the maximally potentiated twitch is, however, significantly prolonged by a tetanus at any temperature in the range 3½ to 30°C in the present experimental studies (Figure 6.5). In the scheme of Close and Hoh (1968a), there would probably be little or no change in T_c, $T_{\frac{1}{2}r}$ and dP/dt. The maximum rate of tension development was not measured, and it is doubtful whether the discrepancy is a result of stimulation of the muscle nerve, and not the muscle directly. Connolly, Gough and Winegrad (1971), by stimulating the sartorius muscle of the frog directly, observed an increase in both T_c and dP/dt in the maximally potentiated twitch. The meaning of the term 'degree of activation' is rather flexible; in a muscle it would be proportional to the number of active fibers (Close and Hoh, 1968a) whereas in a single fiber it would probably be a measure of the capacities to bear a load and to shorten. The 'degree of activation' may be related to the intrinsic speed of shortening in a particular muscle or muscle fiber at a particular temperature. In light of the experimental evidence presented here or elsewhere (for example, Connolly, Gough and Winegrad, 1971), it is certain that the change in the time course of a twitch after a tetanus can be explained in some other way. It does not conflict with Close's (1965, 1968a) suggestion that the intrinsic speed of shortening is not changed by a tetanus and is also independent of the 'degree of activation'.

In relation to the model, it is obvious that decreasing β

gives a twitch which resembles the maximally potentiated twitch observed experimentally (Figure 4.1). The significance of β, the rate of re-uptake of Ca^{++}, is discussed in more detail later in reference to changes of the relaxation phase of an isometric twitch after a tetanus. It is difficult to speculate about the effect of temperature change on the parameters of the model. Reducing β alone does give a twitch whose $T_{\frac{1}{2}r}$ is greatly prolonged, but T_c is not significantly increased, and there is a very small increase in dP/dt (Figure 4.1), whereas the time course of the twitch of a real muscle is greatly prolonged by a decrease in temperature. The other rate constant, α, which also governs the time course of the activation factor, has a relatively small effect on the twitch.

The rate of making of cross-bridges, in a loose sense, can be related to the intrinsic speed of shortening, which is decreased when temperature is lowered. From a look at the separate effects of β and f_1 (Figures 4.3 and 4.4 respectively), it is expected that reducing both β and f_1 would give a response quite similar to that of a real muscle with a decrease in temperature. However, temperature affects all the parameters in a complicated manner and experimental studies seem to be the most rewarding approach in this regard.

In studying the effects of tetanus on the characteristics of the isometric twitch, Connolly, Gough and Winegrad (1971) observed that the potentiation was not maximal until some time after the tetanus. He suggested that this is due to the net effect of two processes. The fast process is responsible for the delayed increase in P_t and a greater T_c after the tetanus. This is also identified with a

fast-diminishing inhibition: during the tetanus, the Ca^{++} stored in the terminal cisternae is depleted. As a result, the amount of calcium released immediately after a tetanus by a stimulus is smaller. This would result in a smaller P_t. This inhibition is alleviated when the calcium store is replenished by relocation of Ca^{++} from the longitudinal tubules and intermediate cisternae portions of the sarcoplasmic reticulum after re-uptake. These authors analysed the change in P_t, dP/dt and T_c and found that these characteristics followed a time course and had a Q_{10} similar to that observed for the calcium movement in the sarcoplasmic reticulum after a tetanus. The fast-diminishing inhibition results from the depletion of calcium and from its fast replenishment.

In the studies reported here, by stimulating the muscle nerve, similar changes were observed for P_t. In the temperature range $3\frac{1}{2}°$ to 25°C, P_t usually increased nearly linearly to a maximum and then started to decay exponentially. This is expected if P_t indicates the sum of a fast-decaying and a slow-decaying process. At higher temperatures, the potentiation reached a maximum in the first few seconds after a tetanus. Analysis showed that the rising phase has a Q_{10} of 2.7 compared to 1.4 as found by Connolly, Gough and Winegrad (1971). It takes about 15 sec to reach its peak at 20°C.

The falling phase of the potentiation is associated with a slowly-decaying potentiation process immediately following a tetanus. These authors, however, reported that the underlying processes responsible were very complex but did not elaborate on their exact nature. In the studies reported here, the slow phase as measured from

the falling phase of potentiation (using P_t) has a half-time of about 1½ min at 20°C and a Q_{10} of 2.9.

The amount of calcium released from the terminal cisternae is large during the tetanus. This large amount would tend to saturate the process of re-uptake by the sarcoplasmic reticulum. It is then not unreasonable to suggest that this slowly-decaying potentiation is due to a decrease in the rate of re-uptake (β) of Ca^{++} to a minimum value after the tetanus. The fact that $T_{\frac{1}{2}r}$ always has the greatest value immediately after a tetanus supports this view.

The changes in P_t and $T_{\frac{1}{2}r}$ after a tetanus can also be associated with some of the parameters of the model. If C_0 is reduced, the peak tension is also reduced as observed in the experiments (Figure 4.1). Reducing β in the model prolongs the relaxation phase but also increases P_t (Figure 4.1). P_t is, however, mostly dependent on C_0 whereas the relaxation phase is governed mainly by β. At higher temperatures, the Ca^{++} store is continuously and quickly replenished, even during the course of the tetanus. As a result, C_0 may not change significantly after the tetanus; whereas since the return of β to its pretetanic value is a much slower process, its effect is exaggerated, leading to a maximal potentiation almost immediately after the tetanus. The decay of this potentiation would be a total replenishment of the calcium store, with β increasing from its post-tetanus value. This process would take more than 15 minutes at lower temperatures.

(ii) Contraction Time, T_c and Half-Relaxation Time, $T_{\frac{1}{2}r}$.

The reciprocals of T_c and $T_{\frac{1}{2}r}$ are referred to as the contraction-rate and relaxation-rate respectively. Both the contraction and relaxation rates are observed to be minimal after the tetanus but the time course of recovery is more variable than P_t. From the slopes obtained by fitting the points with straight lines in Figure 6.5, the ratio contraction-rate/relaxation-rate (equal to $T_{\frac{1}{2}r}/T_c$) equals 1.5 and 2 for the 'control' and the maximally potentiated twitch. The ratio $T_{\frac{1}{2}r}/T_c$ is, in fact, greater for the potentiated twitch at higher temperatures, whereas at lower temperatures, this ratio does not change appreciably. Supporting evidence was also obtained from the frequency response measured after a tetanus, as will be obvious in the discussion later.

7.6 *Effects of Tetanus and Temperatures on the Frequency Response of the Isometric Muscle.*

The representation of the muscle in these studies by a second-order system is extremely good, as shown by analysis in the frequency domain. This is true whether the Fourier transform was computed from a twitch (Figure 6.6) or when the frequency response was obtained by random stimulation (Figure 6.7). The coherence obtained in the latter analysis is high (> .8 for most frequencies). This shows that with the low rate of stimulation used (mean rate = 5/sec), the muscle responds quite linearly. At higher mean rates, the coherence becomes more variable (Stein, French, Mannard and Yemm, 1972).

The percentage increase in zero-frequency gain G_0 is greater in the frequency response obtained after a short tetanus of a few seconds duration at lower temperatures; here the fusion frequency is lower while the muscle does not fatigue quickly. As a result, potentiation lasts longer and gives a larger G_0 in the frequency response. At higher temperatures, G_0 is more variable. Sometimes a decrease is observed although a small increase is most common. This seems paradoxical because potentiation occurs at all temperatures and should give a larger G_0. In the studies on the effect of tetanus on the characteristics of the isometric twitch, it was, however, observed that P_t may be depressed after a potentiation (compared with the pretetanic value). The depression of P_t seems to be related to the smaller G_0 obtained sometimes. This may result from muscle fatigue or from some other unknown causes.

The most consistent changes with temperature are observed in f_n. This is partly because T_c and $T_{\frac{1}{2}r}$ always increased after a tetanus, although P_t sometimes decreased to a value smaller than the 'control' after a potentiation. In fact, a nearly linear relationship (corr. coeff. = .98) is found for f_n in the 'control'. In the 30 sec after a tetanus, f_n is decreased for every temperature but also varies nearly linearly with temperature.

Hutchinson, Koles and Smith (1970) found that in toad myelinated nerve fibers the conduction velocity varies linearly with temperature. If f_n indicates the speed of response of the muscle, it seems that at least one neuronal and another mechanical response in the neuromuscular system vary linearly with temperature. Whether

this linearity is important to movement co-ordination in poikilothermous animals at different temperatures is at present unknown.

At lower temperatures, ζ was usually somewhat greater than 1, but a tetanus did not seem to change its value appreciably. At higher temperatures, the effect of tetanus was very marked and ζ was always increased. Since at any temperature a tetanus increased both T_c and $T_{\frac{1}{2}r}$, the above findings seem paradoxical. This can, however, be explained as follows. If the impulse response is calculated for a second-order model with different values of ζ, the measured ratio $T_{\frac{1}{2}r}/T_c$ can be related to ζ. For example, it is found that $T_{\frac{1}{2}r}/T_c = 1.9$ and 1.68 for $\zeta = 1.2$ and 1 respectively. In other words, ζ depends on the ratio $T_{\frac{1}{2}r}/T_c$, and not their absolute values. At all temperatures, T_c, $T_{\frac{1}{2}r}$ and the ratio $T_{\frac{1}{2}r}/T_c$ are increased by a tetanus, but at higher temperatures, it was observed that a tetanus brought about a disproportional increase in $T_{\frac{1}{2}r}$ relative to T_c, and therefore ζ is increased markedly. Because f_n is observed to increase with a temperature increase, in both the 'control' and the 'post-tetanus' response, it is in a sense increasing in proportion to both $T_{\frac{1}{2}r}$ and T_c, and is thus a good indicator of the 'sluggishness' of the response of the muscle.

In conclusion, the second-order representation of the muscle and the model studied here has been demonstrated to be extremely good, as was also reported in mammalian muscle (Stein, French, Mannard and Yemm, 1972; Mannard and Stein, 1972). These authors suggested that, despite the many rate constants governing the chemical and

mechanical processes underlying activation and contraction, only two are rate-limiting. In the work reported here, it was also found that among all the parameters in the model, two seem to affect the characteristics of the isometric twitch most significantly. These are f_1, the rate of making of cross-bridges, and β, the rate of re-uptake of calcium by the sarcoplasmic reticulum. These rates also seem to determine and can be used to explain to some extent the functional and structural differences found in real muscles and the changes of the tension response under different physiological conditions, such as after a tetanus and temperature change. Additional rate constants seem to determine the changes in β after a tetanus and the movement of Ca^{++} in the sarcoplasmic reticulum, and between the latter structure and the myofilament space. These can only be elucidated by studying the biochemical and biophysical reactions underlying the release, binding and re-accumulation of calcium. Other reactions involving the utilization of ATP, which is closely related to the chemical process of calcium activation can be included in the model. The difference in activation-contraction coupling mechanisms observed for fast and slow muscles could also be included in future work.

REFERENCES.

Anderson-Cedergren, E. (1959) Ultrastructure of motor end plate and sarcoplasmic components of mouse skeletal muscle fiber as revealed by three-dimensional constructions from serial sections. J. Ultrastruct. Res., suppl. 1, 1-19.

Appleberg, B. and Emonet-Denand, F. (1967) Motor units of the first superficial lumbrical muscle of the cat. J. Neurophysiol. *30*, 154-160.

Armstrong, C.M., Huxley, A.F. and Julian, F.J. (1966) Oscillatory responses in frog skeletal muscle fibers. J. Physiol. *186*, 26-27P.

Barany, M. (1966) ATP-ase activity of myosin correlated with speed of muscle shortening. J. gen. Physiol. *50*, 197-218.

Bell, P.M.G. and Stein, R.B. (1971) A digital stimulator built on modular principles using integrated circuits. J. Physiol. *218*, 5P.

Bennett, H.S. (1955) Modern concepts of the structure of striated muscle. Amer. J. Phys. Med. *34*, 46-47.

Bennett, H.S. and Porter, K.R. (1953) An electron microscope study of sectioned breast muscle of the domestic fowl. Amer. J. Anat. *93*, 61-105.

Birks, R.I. and Davey, D.F. (1969) Osmotic responses demonstrating the extracellular character of the sarcoplasmic reticulum. J. Physiol. *202*, 171-188.

Bozler, E. (1954) Mechanism of relaxation in extracted muscle fibers. Amer. J. Physiol. *167*, 276-283.

Brown, G.L. and von Euler, U.S. (1938) The after-effects of a tetanus on mammalian muscle. J. Physiol. *93*, 39-60.

Brown, M.C., Goodwin, G.M. and Matthews, P.B.C. (1971) Tension changes in extrafusal muscle on small amplitude sinusoidal stretching and their significance for the modelling of the muscle spindle. J. Physiol. *218*, 20-21P.

Burke, R.E. (1967) Motor unit types of cat triceps surae muscle. J. Physiol. *193*, 141-160.

Close, R. (1962) The pattern of activation in the sartorius muscle of the frog. J. gen. Physiol. *46*, 1-18.

Close, R. (1965) The relation between intrinsic speed of shortening and duration of the active state of muscle. J. Physiol. *180*, 542-599.

Close, R.I. (1972) The relations between sarcomere length and characteristics of isometric twitch contractions of frog sartorius muscle. J. Physiol. *220*, 745-762.

Close, R. and Hoh, J.F.Y. (1968a) Influence of temperature on isometric contractions of rat skeletal muscles. Nature *217*, 1179-1180.

Close, R. and Hoh, J.F.Y. (1968b) The after-effects of repetitive stimulation on the isometric twitch contraction of rat fast skeletal muscle. J. Physiol. *197*, 461-477.

Connolly, R., Gough, W. and Winegrad, S. (1971) Characteristics of the isometric twitch of skeletal muscle immediately after a tetanus. J. gen. Physiol. *57*, 697-709.

Cooper, S. and Eccles, J.C. (1930) The isometric responses of mammalian muscles. J. Physiol. *69*, 377-385.

Costantin, L.L. and Podolsky, R.J. (1966) Evidence for depolarization of the internal membrane system in activation of frog semitendinosus muscle. Nature *210*, 483-486.

Costantin, L.L., Podolsky, R.J. and Rice, L.W. (1967) Calcium activation of frog slow muscle fibers. J. Physiol. *188*, 261-271.

Davies, R.E. (1968) A molecular theory of muscle contraction: Calcium-dependent contractions with hydrogen bond formation plus ATP-dependent extensions of part of the myosin-actin cross-bridges. Nature *199*, 1068-1074.

D'Azzo, J.J. and Houpis, C.H. (1966) Feedback Control System Analysis and Synthesis. 2nd Edn., McGraw-Hill, New York.

Desmedt, J.E. and Hainaut, K. (1968) Kinetics of myofilament activation in potentiated contraction: Staircase phenomenon in human skeletal muscle. Nature *217*, 529-532.

Ebashi, S. (1960) Calcium binding and relaxation in the actomyosin system. J. Biochem. (Tokyo) *48*, 150-151.

Ebashi, S. (1961a) Calcium binding activity of vesicular relaxing factor. J. Biochem. (Tokyo) *50*, 236-244.

Ebashi, S. (1961b) The role of 'relaxing factor' in contraction-relaxation cycle of muscle. Prog. Theor. Phys. *17*, Suppl. 35-40.

Ebashi, S., Ebashi, F. and Kodama, A. (1967) Troponin as the Ca^{++} receptive protein in the contractile system. J. Biochem. (Tokyo) *62*, 137-138.

Edman, K.A.P. and Grieve, D.W. (1964) On the role of calcium in the excitation-contraction process of frog sartorius muscle. J. Physiol. *170*, 138-152.

Engel, W.K. and Irwin, R.L. (1967) A histochemical-physiological correlation of frog skeletal muscle fibers. Am. J. Physiol. *213*, 511-518.

Falk, G. and Fatt, P. (1964) Linear electrical properties of striated muscle fibers observed with intracellular microelectrodes. Proc. R. Soc. B*160*, 69-123.

Flitney, F.W. (1971) The volume of the T-system and its association with the sarcoplasmic reticulum in slow muscle fibers of the frog. J. Physiol. *217*, 243-257.

Floyd, K. and Smith, I.C.H. (1971) The mechanical and thermal properties of frog slow muscle fibers. J. Physiol. *213*, 617-631.

Frank, G.B. (1958) Inward movement of calcium as a link between electrical and mechanical events in contraction. Nature *182*, 1800-1801.

Frank, G.B. (1960) Effects of changes in extracellular calcium concentration on the potassium-induced contracture of frog's skeletal muscle. J. Physiol. *151*, 518-538.

Frank, G.B. (1962) Utilization of bound calcium in the action of caffeine and certain multivalent cations on skeletal muscle. J. Physiol. *163*, 255-268.

Franzini-Armstrong, C. and Porter, K.R. (1964) Sarcolemmal invaginations constituting the T-system in fish muscle fibers. J. Cell Biol. *22*, 675-696.

French, A.S. and Holden, A.V. (1971a) Alias-free sampling of neuronal spike trains. Kybernetik *8*, 165-171.

French, A.S. and Holden, A.V. (1971b) Frequency domain analysis of neurophysiological data. Comp. Prog. Biomed. *1*, 219-234.

French, A.S. and Holden, A.V. (1971c) Semi-on-line implementation of an alias-free sampling for neuronal signals. Comp. Prog. Biomed. *2*, 1-7.

Gilbert, D.L. and Fenn, W.O. (1957) Calcium equilibrium in muscle. J. gen. Physiol. *40*, 393-408.

Goffart, M. and Ritchie, J.M. (1952) The effect of adrenaline on the contraction of mammalian skeletal muscle. J. Physiol. *116*, 357-371.

Gordon, A.M., Huxley, A.F. and Julian, F.T. (1966) The variation in isometric tension with sarcomere length in vertebrate muscle fibers. J. Physiol. *184*, 170-192.

Hartree, W. and Hill, A.V. (1921) The nature of the isometric twitch. J. Physiol. *55*, 389-411.

Hasselbach, W. (1964) Relaxing factor and the relaxation of muscle. Prog. Biophys. Biol. *14*, 167-222.

Heilbrunn, L.V. and Wiercynski, F.J. (1947) The action of various cations on muscle protoplasm. J. Cell. Comp. Physiol. *29*, 15-32.

Henneman, E. and Olson, C.B. (1965) Relations between structure and function in the design of skeletal muscles. J. Neurophysiol. *28*, 581-598.

Hess, A. (1967) The structure of vertebrate slow and twitch muscle fibers. Invest. Ophth. *6*, 217-228.

Hess, A. (1970) Vertebrate slow muscle fibers. Physiol. Rev. *50*, 40-62.

Hill, A.V. (1938) The heat of shortening and the dynamic constants of muscle. Proc. R. Soc. B*126*, 136-195.

Hill, A.V. (1949) The abrupt transition from rest to activity in muscle. Proc. R. Soc. B*136*, 399-420.

Hill, A.V. (1951a) The effect of series compliance on the tension developed in the muscle twitch. Proc. R. Soc. B*138*, 325-329.

Hill, A.V. (1951b) The transition from rest to full activity in muscle: The velocity of shortening. Proc. R. Soc. B*138*, 329-338.

Hill, A.V. (1951c) The influence of temperature on the tension developed in an isometric twitch. Proc. R. Soc. B*138*, 349-354.

Hill, A.V. and Macpherson, L. (1954) The effect of nitrate, bromide and iodide on the duration of the active state in skeletal muscle. Proc. R. Soc. B*143*, 81-102.

Hill, D.K. (1968) Tension due to interaction between the sliding filaments in resting striated muscle. The effect of stimulation. J. Physiol. *199*, 637-684.

Hutchinson, N.A., Koles, Z.J. and Smith, R.S. (1970) Conduction velocity in myelinated nerve fibers of *Xenopus laevis*. J. Physiol. *208*, 279-289.

Huxley, A.F. (1957) Muscle structure and theories of contraction. Prog. Biophys. Biophys. Chem. *7*, 255-318.

Huxley, A.F. and Niedergerke, R. (1954) Interference microscopy of living muscle fibers. Nature *173*, 971-973.

Huxley, A.F. and Simmons, R.M. (1971) Proposed mechanism of force generation in striated muscle. Nature *233*, 533-538.

Huxley, A.F. and Taylor, R.E. (1958) Local activation of striated muscles from the frog and the crab. J. Physiol. *144*, 426-441.

Huxley, H.E. (1964) Evidence for continuity between the central elements of the triads and extracellular space in frog sartorius muscle. Nature *202*, 1067-1071.

Huxley, H.E. (1969) The mechanism of muscular contraction. Science *164*, 1356-1366.

Huxley, H.E. and Hanson, J. (1954) Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. Nature *173*, 973-976.

Ishiko, N. and Sato, M. (1957) The effect of calcium ions on electrical properties of striated muscle fibers. Jap. J. Physiol. *7*, 51-63.

Jewell, B.R. and Wilkie, D.R. (1958) An analysis of the mechanical components in frog's striated muscle. J. Physiol. *143*, 515-540.

Jewell, B.R. and Wilkie, D.R. (1960) The mechanical properties of relaxing muscle. J. Physiol. *152*, 30-47.

Julian, F.J. (1969) Activation in a skeletal muscle contraction model with a modification for insect fibrillar muscle. Biophys. J. *9*, 547-570.

Julian, F.J. (1971) The effect of calcium on the force-velocity of briefly glycerinated frog muscle fibers. J. Physiol. *218*, 117-145.

Kelly, E. and Fry, W.J. (1958) Isometric twitch tension of frog skeletal muscle as a function of temperature. Science *128*, 200-202.

Kernell, D. (1966) The repetitive discharge of motoneurons. In "Muscular Afferents and Motor Control. Nobel Symposium I". (Granit, R., ed.). Pp. 351-362, Stockholm: Almquist and Wiksell.

Knox, C.K. (1969) The power spectral density of random spike trains. In "System Analysis Approach to Neurophysiological Problems". (Terzuolo, C.A., ed.). Laboratory of Neurophysiologh, University of Minnesota.

Kuffler, S.W. and Gerard, R.W. (1947) The small-nerve motor system to skeletal muscle. J. Neurophysiol. *10*, 383-394.

Kuffler, S.W., Laporte, Y. and Ransmeier, R.E. (1947) The function of the frog's small-nerve motor system. J. Neurophysiol. *10*, 395-408.

Kuffler, S.W. and Vaughan-Williams, E.M. (1953) Properties of the 'slow' skeletal muscle fibers of the frog. J. Physiol. *121*, 318-340.

Lannergren, J. and Smith, R.S. (1966) Types of muscle fibers in toad skeletal muscle. Acta physiol. scand. *68*, 263-274.

Lee, K.S., Ladinsky, H., Choi, S.J. and Kasuya, Y. (1966) Studies on the *in vitro* interaction of electrical stimulation and Ca^{++} movement in sarcoplasmic reticulum. J. gen. Physiol. *49*, 689-715.

Mannard, A. and Stein, R.B. (1972) Determination of the frequency response of isometric soleus muscle in the cat using random stimulation. J. Physiol., submitted for publication.

Marsh, B.B. (1952) The effects of adenosine triphosphate on the fiber volume of a muscle homogenate. Biochim. Biophys. Acta *9*, 247-260.

Matthews, P.B.C. (1959) The dependence of tension upon the tension in the stretch reflex of the soleus muscle of the decerebrate cat. J. Physiol. *147*, 521-546.

Nachmias, V.T. and Padykula, H.A. (1958) A histochemical study of normal and denervated red and white muscles of the rat. J. Biophys. Biochem. Cytol. *4*, 47-54.

Ogata, K. (1970) Modern Control Engineering. Prentice-Hall, Eaglewood Cliffs, New Jersey.

Page, S. (1964) The organization of the sarcoplasmic reticulum in frog muscle. J. Physiol. *175*, 10-11P.

Page, S.G. (1968) Structures of the sarcoplasmic reticulum in vertebrate muscle. Brit. med. Bull. *24*, 170-173.

Partridge, L.D. (1965) Modifications of neural output signals by muscles; a frequency response study. J. appl. Physiol. *20*, 150-156.

Partridge, L.D. (1966) Signal-handling characteristics of load-moving skeletal muscle. Am. J. Physiol. *210*(5), 1178-1191.

Peachey, L.D. (1961) Structure and function of slow striated muscle. In "Biophysics of Physiological and Pharmacological Action". Pp. 391-411. (Shanes, A.M., ed.). Washington: American Association for Advanced Science.

Peachey, L.D. (1965) Transverse tubules in excitation-contraction coupling. Fed. Proc. *24*, 1124-1134.

Peachey, L.D. (1968) Muscle. Ann. Rev. Physiol. *30*, 401-440.

Podolsky, R.J. and Costantin, L.L. (1964) Regulation by calcium of the contraction and relaxation of muscle fibers. Fed. Proc. *23*, 933-939.

Podolsky, R.J., Nolan, A.C. and Zaveler, S.A. (1969) Cross-bridge properties derived from muscle isotonic velocity transients. Proc. Nat. Acad. Sci., U.S.A. *64*, 504-511.

Poppele, R.E. and Terzuolo, C.A. (1968) Myotatic reflex: its input-output relation. Science, N.Y. *159*, 743-745.

Porter, K.R. (1956) The sarcoplasmic reticulum in muscle cells of *Amblystoma* larvae. J. Biophys. Biochem. Cytol. *2*, 163-170.

Porter, K.R. (1961) The sarcoplasmic reticulum. Its recent history and present status. J. Biophys. Biochem. Cytol. *10*, No. 4 Suppl., 219-226.

Porter, K.R. and Pallade, G.E. (1957) Studies on the endoplasmic reticulum. III. Its form and distribution in striated muscle cells. J. Biophys. Biochem. Cytol. *3*, 269-300.

Portzehl, H., Caldwell, P.C. and Ruegg, J.C. (1964) The dependence of contraction and relaxation of muscle fibers from the drab, *Maia squinado*, on the internal concentration of free calcium ions. Biochim. Biophys. Acta. *79*, 581-591.

Pringle, J.W.S. (1965) Locomotion: flight. In "The Physiology of Insecta. (Rockstein, M., ed.). Academic Press, New York and London *2*, 283 329.

Pringle, J.W.S. (1967) The contractile mechanisms of insect fibrillar muscle. Prog. Biophys. Molec. Biol. *17*, 1-60.

Rack, P.M.H. and Westbury, D.R. (1969) The effects of length and stimulus rate of tension in the isometric cat soleus muscle. J. Physiol. *204*, 443-460.

Ramsey, R.W. and Street, S.F. (1941) Muscle function as studied in single muscle fibers. Biol. Symp. *3*, 9-34.

Ritchie, J.M. and Wilkie, D.R. (1955) The effect of previous stimulation on the active state of muscle. J. Physiol. *130*, 488-496.

Romanul, F.C.A. (1964) Enzymes in muscle. I. Histochemical studies of enzymes in individual muscle fibers. Arch. Neurol. *11*, 355-368.

Rosenthal, N.P., McKean, T.A., Roberts, W.J. and Terzuolo, C.A. (1970) Frequency analysis of stretch reflex and its main subsystems in triceps surae muscle of the cat. J. Neurophysiol. *33*, 713-749.

Sandow, A. (1965) Excitation-contraction coupling in skeletal muscle. Pharmac. Rev. *17*, 265-319.

Smith, D.S. (1966) The organization and function of the sarcoplasmic reticulum and T-system of muscle cells. Prog. Biophys. Molec. Biol. *16*, 107-142.

Smith, R.S. and Lannergren, J. (1968) Types of motor units in the skeletal muscle of *Xenopus laevis*. Nature *217*, 281-283.

Stein, J.M. and Padykula, H.A. (1962) Histochemical classification of individual skeletal muscle fibers of the rat. Am. J. Anat. *110*, 103-124.

Stein, R.B. and French, A.S. (1970) A flexible neural analog using integrated circuits. I.E.E.E. Trans. Bio-Med. Engng. *17*, 248-253.

Stein, R.B., French, A.S., Mannard, A.C. and Yemm, R. (1972) New methods for analysing motor function in man and animals. Brain Res. *40*, 187-192.

Tasaki, I. and Mizutani, K. (1944) Comparative studies on the activities of the muscle evoked by two kinds of motor nerve fibers. Jap. J. Med. Sci. Biophys. *10*, 237-244.

Thorson, J. and White, D.C.S. (1969) Distributed representations for actin-myosin interaction in the oscillatory contraction of muscle. Biophys. J. *9*, 360-390.

Walker, S.M. (1949) Potentiation of twitch tension and prolongation of action potential induced by reduction of temperature in rat and frog muscle. Am. J. Physiol. *157*, 429-435.

Walker, S.M. (1951) Tension and extensibility changes in muscles suddenly stretched during the twitch response. Am. J. Physiol. *164*, 238-247.

Watanabe, S. and Sleater, W., Jr. (1957) EDTA relaxation of glycerol-treated muscle fibers, and the effects of magnesium, calcium and manganese ions. Arch. Biochem. Biophys. *68*, 81-101.

Weber, A. (1966) Energized calcium transport and relaxing factors. Current Topics in Bioenergetics *1*, 203-254.

Weber, A. and Winicur, S. (1961) The role of calcium in the super-precipitation of actomyosin. J. Biol. Chem. *236*, 3198-3202.

Winchester, B.T. (1969) Mechanical Properties of Denervated Rat Diaphragm Muscle. M.Sc. Thesis, University of Alberta.

Winegrad, S. (1965a) Autoradiographic studies of intracellular calcium in frog skeletal muscle. J. gen. Physiol. *48*, 455-479.

Winegrad, S. (1965b) The location of muscle calcium with respect to the myofibrils. J. gen. Physiol. *48*, 997-1002.

Winegrad, S. (1968) Intracellular calcium movements of frog skeletal muscle during recovery from tetanus. J. gen. Physiol. *51*, 65-83.

Winegrad, S. (1970) The intracellular site of calcium activation of contraction in frog skeletal muscle. J. gen. Physiol. *55*, 77-88.

Wuerker, R.B., McPhedran, A.M. and Henneman, E. (1965) Properties of motor units in a heterogeneous pale muscle (M. gastrocnemius) of the cat. J. Neurophysiol. *28*, 85-99.